JOURNAL

OF

HELMINTHOLOGY

Edited by

R. T. LEIPER, M.D., D.Sc., F.R.S.,

Professor of Helminthology in the University of London.

VOL. IV., 1926.

Published from the
London School of Hygiene and Tropical Medicine,
23, Endsleigh Gardens,
London,
W.C. 1.

CONTENTS OF VOLUME IV.

Contents.

No. 3 (AUGUST, 1926).

Nos. 4-5 (NOVEMBER, 1926).

Journal of Helminthology, Vol. IV., *No.* 1, *March*, 1926, *pp.* 1-6.

On the Ascaris from Sheep.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Introduction.

Worms belonging to the genus Ascaris occur occasionally in the small intestine of sheep and lambs and have been reported both in Europe and America. They have been considered by some to belong to a distinct species, namely *Ascaris ovis* Rudolphi, and by others to be identical with *Ascaris lumbricoides* Linnæus. The question of their specific identity is an interesting one and could not be considered as definitely settled one way or the other and though possibly the majority of helminthologists would subscribe to the view that the species is *A. lumbricoides* (*vide* Ransom 1911, p.25, and Ransom and Foster 1920, p. 30), others hold a different view; in fact Neuveu-Lemaire (1923) has recently published a paper in which he describes a single specimen of Ascaris from a goat as *Ascaris ovis*.

The writer has had an opportunity of examining a numerous and well preserved collection of adult Ascarids obtained from sheep at an abattoir in London at various times in the early months of 1925. In these worms the lips surrounding the mouth have papillæ arranged in the manner characteristic of *A. lumbricoides*, and the male tail in all the specimens examined possessed caudal papillæ to the same number and having the same distribution as those of the male tail of *A. lumbricoides*.

In order to settle the question as completely as possible two worms, one of each sex, were obtained from the collection described by Neumann (1884) as *Ascaris ovis*, preserved at the École Nationale Vétérinaire, Toulouse, France. These were carefully examined to determine the

characters of the head papillæ and the caudal papillæ of the male with the result that in these points they were found to be entirely characteristic of *A. lumbricoides*. These observations have led the writer to the conclusion that there is no distinct species *A. ovis* and that the worms occurring occasionally in sheep are morphologically identical with *A. lumbricoides*.

The best thanks of the writer are due to Director Ch. Besnoit and Prof. A. Martin, of the École Nationale Vétérinaire, Toulouse, for their kindness in sending him the two worms from Neumann's collection.

Morphology.

The worms obtained from sheep in London were preserved in 10 per cent. formalin whilst perfectly fresh and are therefore in very good condition. They are, on the whole, much slenderer in appearance than mature specimens of *A. lumbricoides* taken from man and pigs.

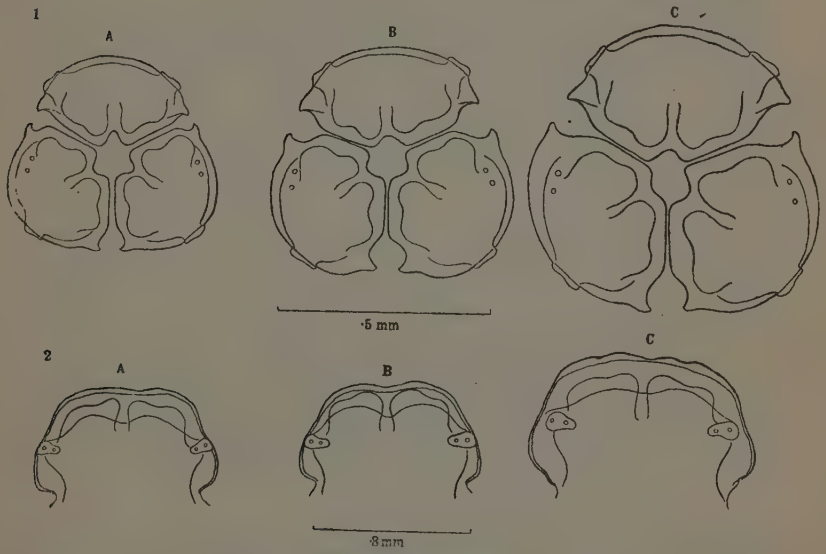
Measurements were made of 22 males and 49 females. The former ranged in length from 10 to 12·7 cm., whilst the latter, except for two small specimens measuring 7·8 and 9·8 cm. respectively, formed a well graduated series ranging from 11 to 19·5 cm. in length. The breadth varied from 1·5 to 2 mm. for the shorter forms and 3 to 4 mm. for the longer forms.

Lip characters. It is generally recognised that the shape of the lips surrounding the mouth and the number and disposition of the papillæ on them are important for the determination of Ascaris species and the writer has paid particular attention to these structures. Neumann (l.c., pp. 383-4) says that in the worms examined by him, 10 males and 19 females, the three lips are of the same type as those in *A. lumbricoides*. The dorsal bears two papillæ and the sub-ventrals bear a single papilla each situated *in the middle of its convex surface.* The last few words are italicised because though both Neumann and Neuveu-Lemaire (l.c.) figure the papillæ of the sub-ventral lips as occupying practically a median position on the convex face of each lip, these papillæ are not so situated but are displaced somewhat towards the ventral line as can be seen from Fig. 1, A, B and C.

In addition to these large papillæ each sub-ventral lip carries two distinct, simple papillæ seen in face view occupying a position close

to the ends of the transverse axis of the head. As the drawings show, these papillæ have been found in the worms from Toulouse and London sheep as well as in the pig material.

Neumann says that a difference between *A. ovis* and *A. lumbricoides* consists in the situation of the lip papillæ. They occupy in the former the middle of the height of the lip and are consequently further from the base than in *A. lumbricoides*. It is of interest to note in passing



Ascaris lumbricoides.

Fig 1.—End-on view of head showing situation of lip papillæ, A, from sheep, Neumann's material, B, from sheep, London material C, from pig.

Fig. 2.—Dorsal lips showing papillæ, dentigerous ridges and pulps, A, from sheep, Neumann's material, B, from sheep, London material, C, from pig.

that practically the same form of words to describe their position is used by both Neumann and Neuveu-Lemaire; the former says they are "moins rapprochées de la base que dans *A. lumbricoides*," and the latter "moins rapprochées de la base de lèvres que chez *A. lumbricoides*."

Drawings of the dorsal lip of three worms, two from sheep and one from pig, have been made to the same scale and are shown in Fig. 2,

A, B and C. It is evident from these that the papillæ in the case of the worm from the Toulouse sheep have the same relative position as those in the worm from the London sheep and that both of these are essentially similar, in respect of the papillæ, to the worm from the pig. Their position relative to the height of the lip is the same in all three cases and no importance can therefore be attached to the suggestion that here was a point of specific difference between the Ascaris from sheep and from the pig.

Male tail. Fig. 3, A, B and C, are drawings of the ventral surface of the tips of the male tail, A from the Toulouse specimen, B from the London sheep material, and C from pig material. In all three cases the post-anal papillæ are the same in number and have the same arrangement, namely, two pairs with double pulps, the anterior pair being the larger, and three pairs of much smaller ones with single pulps, each set of three arranged somewhat in the form of a flattened triangle with the apex directed towards the longitudinal axis of the worm. The lateral and pre-anal papillæ are also similarly arranged in all three cases. The line of the post-anal papillæ is continued forwards on each side of the body as a single row of four papillæ which is followed by a double row extending for some distance anteriorly. The anterior lip of the cloaca is raised into a cushion-like pad in all the worms examined. Neumann's drawing of the male tail is inaccurate and unsatisfactory ; he shows three single post-anal papillæ on either side whilst the pre-anal ones are represented as a single row on each side of the body.

Eggs. A large number of eggs were taken from the uteri of five fairly large females which occurred along with some male worms in one collection. These were found to have the same appearance as the eggs of *A. lumbricoides* from man and pig, *i.e.*, the outer shell was covered with a gelatinous envelope very coarsely mamillated. The contents of many of the eggs looked perfectly normal and as though capable of undergoing development so they were transferred to 2 per cent. formalin in a shallow layer in Petri dishes and were incubated at 24° C. The liquid was aerated daily and after 10 to 14 days about 30 per cent. of the eggs in the culture had undergone development, and contained active embryos. This point is of interest in view of the fact that Ransom and Foster (l.c.p. 29 and 30) say that : " Apparently in no case has a

fully developed female *Ascaris* containing well-formed eggs been found in sheep. The worms have always been apparently underdeveloped." The present results show that *A. lumbricoides* can attain sexual maturity in the sheep as a host and that the eggs can become fertilised and are capable of carrying on the race.

A few of the embryonated eggs were fed to a mouse, but the larvæ liberated from these were not sufficient to produce any symptoms of pneumonia, and after 8 days the animal was chloroformed and opened up. The lungs were carefully teased up in saline and 10 partially developed larval ascarids were found ranging in length from 0·52 to 0·92 mm.

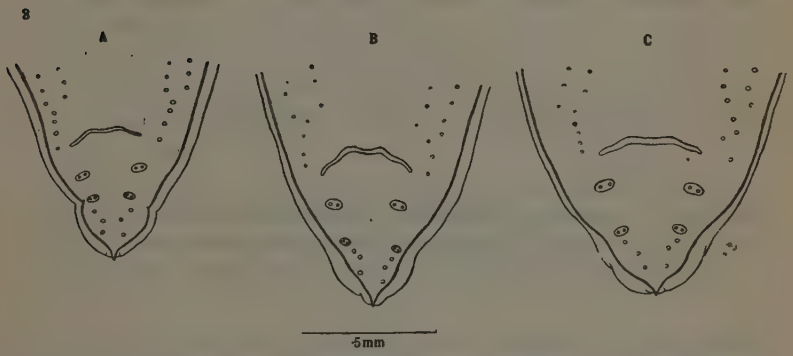


Fig. 3.—Tips of male tail in ventral view showing arrangement of caudal papillæ A, from sheep, Neumann's material, B, from sheep, London material, C, from pig.

Both head and tail characters as described and figured above agree in all particulars with the findings of Thornton (1924) who compared *Ascaris lumbricoides* from man, pig and chimpanzee, and came to the conclusion that the worms from these three hosts are morphologically indistinguishable. Baylis and Daubney (1922) examined Ascaris from man, pig, orang-outang and Indian squirrels, and reached the conclusion

that in all these cases the species was *A. lumbricoides*. Schwartz (1925) records the occurrence of a large number of *A. lumbricoides* in the small intestine of a calf slaughtered at Illinois, U.S.A., in May 1925, this being the first authentic record of the species from a bovine host.

As already stated the writer is satisfied that the Ascaris of sheep is also *A. lumbricoides*; the list of hosts therefore for this parasite at the present time is as follows :—man, chimpanzee, orang-outang, pig, sheep, cattle and squirrels.

REFERENCES.

BAYLIS, H. A., and DAUBNEY, R., 1922.—" Report on the Parasitic Nematodes in the Collection of the Zoological Survey of India." *Memoirs of the Indian Museum*, vol. VII., No. 4, pp. 266-7. Calcutta.

NEUMANN, M. G., 1884.—" Sur l'Ascaride du Mouton (*Ascaris ovis* Rudolphi)." *Revue Vétérinaire*, pp. 382-386. Toulouse.

NEVEU-LEMAIRE, M., 1923.—" Presence *d'Ascaris ovis* chez le Chevreuil (*Capreolus capreolus*)." *Annales de Parasitologie*, T. 1, pp. 265-268. Paris.

RANSOM, B. H., 1911.—" The Nematodes Parasitic in the Alimentary Tract of Cattle, Sheep and other Ruminants." Bureau of Animal Industry, Bull. 127, U.S.A., pp. 24-25. Washington.

RANSOM, B. H., and FOSTER, W. D., 1920.—" Observations on the Life History of *Ascaris lumbricoides*." Bull. 817, U.S. Dept. of Agric., pp. 29 and 30. Washington.

SCHWARTZ, B., 1925.—" Occurrence of Ascaris in Cattle in the United States." *The North American Veterinarian*, vol. VI. (11), Nov., pp. 24-30. Chicago.

THORNTON, H., 1924.—" The Relationship between the *Ascarids* of Man, Pig and Chimpanzee." *Ann. Trop. Med. and Parasit.*, vol. XVIII., No. 1, pp. 99 and 100. Liverpool.

Journal of Helminthology, Vol. IV., *No.* 1, *March*, 1926, *pp.* 7-12.

Helminths of Wild Birds found in the Aberystwyth Area.

By E. Aneurin Lewis, M.Sc.

(*Demonstrator in Zoology, University College of Wales, Aberystwyth, and Field Officer to the Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

In investigating the occurrence of helminthic parasites of wild birds it must be borne in mind that birds are migratory animals so that their parasites may be carried over a wide area. Also it is known that the intermediate hosts of bird helminths are in many cases very widely distributed and that in other cases species nearly related may act as alternative intermediate hosts.

Nicoll (1923) has given a detailed account of the distribution of Trematode parasites in relation to the migration of birds. His paper has been freely used by the writer and references are made to his work in the following list.

Among the present records attention may be called to the Nematode *Subulura suctoria* from a nightjar *Caprimulgus europæus* Linn. shot at Aberystwyth. *S. suctoria* has not been recorded from the British Isles before. It occurs, however, in nightjars in Egypt, where it is common in fowls, and as it is well known that nightjars spend most of their time in Africa it would appear very probable that the bird in question may have migrated to this area from Egypt.

No previous records of helminthic parasites of birds have been published for this district.

TREMATODA.

Genus: *ECHINOSTOMA* Rud., 1809.

Echinostoma paraulum Dietz, 1909.

This species commonly occurred in the rectum of the whooper swan *Cygnus cygnus* Linn. and was found in large numbers in five individual

birds. The birds were shot in the harbour mouth at Aberystwyth where they are often found in the winter months. Nicoll records this species as from the intestine of the whooper swan.

ECHINOSTOMUM REVOLUTUM (Froelich, 1802).
Syn. *E. echinatum* (Zeder, 1803).

A common parasite of the small intestine and rectum of the whooper swans found at Aberystwyth.

This Echinostome was found by Nicoll in the rectum of the whooper swan.

Genus: *HIMASTHLA* Dietz, 1909.

HIMASTHLA ELONGATA (Mehlis, 1831).

Large numbers of this worm were obtained from the small intestine of a herring-gull *Larus argentatus* Pantoppidan.

Genus: *ECHINOCHASMUS* Dietz, 1909.

ECHINOCHASMUS COAXATUS Dietz, 1909.

Three specimens of this Trematode were found in the intestine of a bittern *Botaurus stellaris* Linn. Nicoll (1923) does not record this as occurring in the Bittern, but finds it present in the white stork *Ciconia ciconia* Linn., the great crested grebe *Podiceps cristatus* Boddært and the red-necked *Podiceps griseigena* Boddært.

Genus: *PARYPHOSTOMUM* Dietz, 1909.

PARYPHOSTOMUM RADIATUM (Duj., 1845).

A very common parasite of the small intestine of cormorants *Phalacrocorax carbo* Linn.

These birds are found in large numbers along the cliffs of the sea-shore.

Genus: *PETASIGER* Dietz, 1909.

PETASIGER EXÆRATUS Dietz, 1909.

Enormous numbers of this worm were found in the stomach, and small intestine of cormorants.

Genus: *PARORCHIS* Nicoll, 1907.

PARORCHIS ANCANTHUS Nicoll, 1907.

Only one specimen was obtained from the small intestine of a herring-gull.

Genus: *LEPODERMA* Looss, 1899.

LEPODERMA CIRRATUM (Rud., 1802).

Fifteen specimens were collected from the small intestine of a blackbird *Turdus merula* Linn.

Nicoll does not record this parasite from the blackbird.

Genus: *LYPEROSOMUM* Looss, 1899.

LYPEROSOMUM LONGICAUDA (Rud., 1809).

Syn. *Dicrocœlium macrourum* (Rud., 1819).

This species was found in the liver of a blackbird. Nicoll found it in the gall bladder also.

LYPEROSOMUM sp.

About six specimens of this parasite were found in the small intestine of a whooper swan, and, at present, the writer is doubtful as to the species.

Genus: *CRYPTOCOTYLE* Lühe, 1899.

CRYPTOCOTYLE CONCAVA (Creplin, 1825).

Large numbers of this parasite were obtained from the small intestine of the the black-necked grebe, *Podiceps nigricollis* Brehm.

CRYPTOCOTYLE LINGUA (Creplin, 1825).

Enormous numbers of this parasite were found in the small intestine of a herring-gull.

Genus: *SPELOTREMA* Jägerskiöld, 1901.

SPELOTREMA SIMILE Jägerskiöld, 1900.

This parasite was very common, and occurred in large numbers in the small intestine of the herring-gull.

Genus : *CATATROPIS* Odhner, 1905.

CATATROPIS VERRUCOSA (Froelich, 1789).

Eleven specimens of this monostome were obtained from the cæcum of a whooper swan. The writer did not find it on more than one occasion.

Genus : *STRIGEA* Abildgaard, 1790.

STRIGEA LONGICOLLIS (Rud., 1819).

This, along with the following species, was found in very large numbers in the small intestine of the cormorant. It was found in all cormorants examined.

STRIGEA VARIEGATA (Creplin, 1825).

A very common parasite of the small intestine of the cormorant Both species of Strigea recorded here were, often, too numerous to count.

NEMATODA.

Genus : *PORROCÆCUM* Raillet and Henry 1912.

PORROCÆCUM ENSICAUDATUM (Zeder, 1800).

A very common parasite of starlings, blackbirds, and rooks in this area. It seems that this parasite has not been recorded from rooks before.

PORROCÆCUM SEMITERES (Zeder, 1800).

This parasite does not seem to be so common as *P. ensicaudatum*. The authenticity of the species *P. semiteres* is open to doubt. Baylis (1922), referring to the two species in question points out that there are " two forms which, while possibly hardly more than sub-species, are nevertheless, quite distinct and recognisable." He gives four differences, two of which do not hold for the specimens I have examined. Thus he records to *P. semiteres :* ' (1) Presence of conspicuous lateral alæ, and . . . (4) the length of the spicules of the male, those of *P. semiteres* ·77 to ·8 mm., while those of *P. ensicaudatum* measure only ·62 to ·63 mm." I have seen *P. ensicaudatum* (of Baylis' description) with spicules of ·85 mm.—equal to the spicules of *P. semiteres* (according to Baylis) ; and I have also seen *P. semiteres* (with spicules

of ·78 mm.) with no cervical alæ. In addition to this, I have examined specimens possessing cervical alæ and which have spicules of ·6 to ·65 mm.

PORROCÆCUM SPIRALE (Zeder, 1803).

Three specimens of this parasite were obtained from the small intestine of a Barn Owl (*Flammea flammea* Linn.)

Genus: *CONTRACÆCUM* Raillet and Henry, 1912.

CONTRACÆCUM SPICULIGERUM (Rud., 1809).

A very common parasite in the stomach of Cormorants. It has been found in all Cormorants examined.

Genus: *SUBULURA MOLIN, 1860.*

SUBULURA SUCTORIA Molin, 1860.

This parasite was found in large numbers in the cæcum of a Nightjar captured at Aberystwyth. One Nightjar only was examined.

Genus: *SYNGAMUS* Siebold, 1836.

SYNGAMUS TRACHEALIS (Montagu, 1811).

This parasitic roundworm was found in the windpipe of starlings, thrushes and jays. In the starlings, it was very common; it was found also in thrushes on three occasions and once in a jay.

REFERENCES.

BAYLIS, H. A., 1920.—On the Classification of the Ascaridæ. *Parasitology*, vol. XII., No. 3, pp. 253-264.

BAYLIS, H. A., 1922.—Notes on Two species of Porrocæcum from Birds *P. ensicaudatum* (Zeder 1800) and *P. semiteres* (Zeder 1800). *Ann. Mag. Nat. Hist.* (9), vol. IX.

BLANCHARD, R., 1895.—Animaux parasites. *Bull. Soc. Zool. France*, vol. XX., p. 217.

Dietz, E., 1909.—Die Echinostomiden der Vögel. *Zool. Angeig.*, vol. xxxix., pp. 180-192.

Dietz, E., 1910.—Die Echinostomiden der Vögel. *Zool. Jahrb. Syst.*, vol. xii., Suppl., pp. 265-512.

Lühe, M., 1909.—Parasitische Plattwurmer. Trematodes. *In Die Susswasserfauna Deutschlands.*, vol. xvii.

Megnin, P., 1883.—On the Gapes Disease of Gallinaceous Birds, etc., pp. 1-22.

Nicoll, William, 1923.—A Reference List of the Trematode Parasites of British Birds. *Parasitology*, vol. xv., No. 2, pp. 151-202.

Ortlepp, R. J., 1923.—The Life History of *Syngamus trachealis* (Montagu) v. Siebold, the Gape Worm, of Chickens. *Journal of Helminthology*, vol. i., pp. 119-140.

Waite, R. H., 1920.—Earthworms—the Important Factor in the Transmission of Gapes in Chickens. *Maryl. State Coll. Agric. Bull.* 234, pp. 103-118.

Walker, H. D., 1886.—The Gape Worm of Fowls (*Syngamus trachealis*), the Earthworm (*Lumbricus terrestris*), its original Host, etc. *Bull. Buff. Soc. Nat. Sci.*, vol. v., No. 2, pp. 47-71.

Journal of Helminthology, Vol. IV., *No.* 1, *March*, 1926, *pp.* 13-22.

Observations on the Genus *Echinococcus* Rudolphi, 1801.

By Thomas W. M. Cameron M.A., B.Sc., Ph.D., M.R.C.V.S.

(*Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

Tænia echinococcus has always been recognised as one of the most important tapeworms of carnivores, both from the human and the economic point of view. Its intermediate stage, or Hydatid, assumes many forms, but most modern workers believe that all these forms—with one possible exception, the alveolar form from Central Europe—are merely the manifestations of a single species.

In 1863, however, Diesing described from the jaguar in South America, a species which he named *Tænia oligartha*, and which although closely related to the older *Tænia echinococcus*, was obviously distinct. The present writer in examining a South American cat, *Felis yaguarundi*, which died at the London Zoological Gardens, collected a number of specimens which appear to belong to Diesing's species. The study of this together with other material in this Department, suggested that a re-examination of the question was desirable.

The Genus *Echinococcus*. The genus Tænia in its modern connotation has rather a large number of species, and is divided by many workers into four genera or sub-genera, as follows :—

(a) Tænia (sens. str.) with *T. solium* of man and the various Tænias of carnivores which have a Cysticercus as the asexual stage. (b) *Tænia-rhynchus* which includes *T. saginata* and a few doubtful forms from man, and which differs from the first only in the absence of hooks from the scolex. This is generally regarded as a sub-genus only. (c) *Multiceps* which includes those Tænias of the dog which have a Cœnurus as the asexual stage. They otherwise do not appear to have any valid generic

differences from Tænia (sens. str., as used above). (d) *Echinococcus* which hitherto has included only one species—*E. granulosus*—the common form from dogs and allied animals in Europe and elsewhere ; and which is characterised mainly by the possession of a Hydatid as the asexual stage.

The present writer is unable to accept the view that the first three forms are valid genera. Their differences are so slight both in morphology and biology that he believes that they should all be referred to the genus Tænia. In the case of Echinococcus, however, not only is the hydatid stage entirely different in its morphology and in the development of the immature scolices, but there are distinct differences in the adult form—in shape of hooks, in size and in the small number of the segments. He believes that this genus is a valid one, distinct from the other Tænias of man and the carnivores.

The type of the genus is *Echinococcus granulosus* (Batsch, 1786) of the dog and allied canidæ. In the genus Echinococcus also, should be included *Tænia oligarthra* Diesing, and possibly some other forms. Unfortunately owing to shortage of material, it has been impossible to make this study as exhaustive as is desirable ; and because of this, the validity of several other forms is left doubtful.

Meggitt (1924) in his classification of the Cestoda, includes in the genus Echinococcus the species *granulosus* and *omissa*. The latter, however, obviously does not belong to this genus and is apparently a lapsus for *oligarthra*. Both these species are described by Luhe (1910) in the same paper and Meggitt probably intended to place the species *oligarthra* into the genus Echinoccocus and by a clerical error wrote *omissa*.

ECHINOCOCCUS OLIGARTHRUS (Diesing, 1863).

T. Oligarthra Diesing, 1863.

E. cruzi Brumpt and Joyeux, 1924.

This species was first described by Diesing from *Felis concolor*, and was later studied by Lühe in 1910. The specimens examined by the present writer were collected from *Felis yaguarundi*. Some were fixed in Picro-Acetic, others in Schaudinn's Solution or in Alcohol.

This is a small form with never more than three segments—one immature, one mature, and one gravid. Its total length is about 1·7 mm.

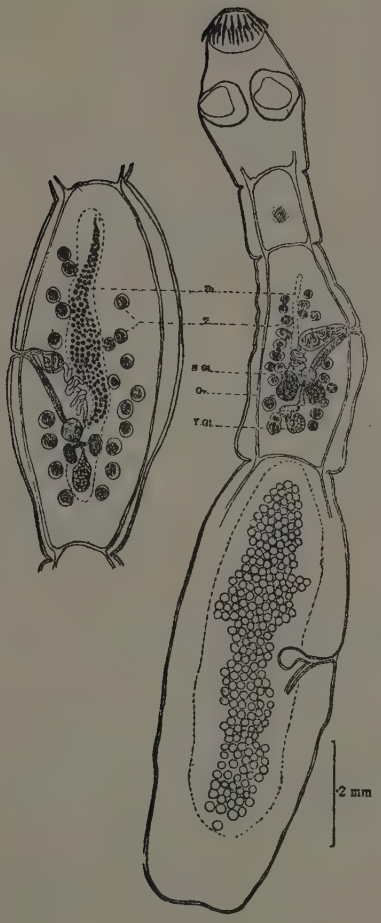


Fig. 1.—*Echinococcus oligarthrus*. Complete adult worm. The single semi-gravid proglottid is drawn from another specimen in order to shew the transitional stage between mature and gravid segments. (Ov. = ovary, Ut. = uterus, T. = testes, Y.Gl. = yolk-gland, S.Gl. = shell gland.)

No specimens were over 2·5 mm. long. It has remarkable powers of movement and in warm saline moves rapidly by alternate waves of contraction and expansion. A similar mode of progression is seen in detached gravid segments.

The *head* is elongated; in a state of expansion—as when allowed to die in saline—it is roughly pentagonal in shape with a blunt apex. The rostellum is very powerful for its size and is armed with two rows of very stout hooks—about 36 to 40 in number.

The *large hooks* are ·045 mm. long (from tip of blade to end of handle). The blade is sharply curved and joins the handle at a distinct notch (Fig. 2, a). The handle is long and undulating, with a blunt end, and is continued in the same line as the blade. The guard is at right angles to the handle and blade, is somewhat elongated and ovate in shape.

The *small hooks* are ·032 mm. long. The blade is shorter and less deeply "hooked" than in the large hooks. The handle is thinner and longer. The guard is comparatively large and prominent, forming an obtuse angle with the handle.

The four suckers are large and circular with a diameter of about ·1 mm.

The immature segment is almost square; the mature twice as long as wide; and the gravid three times as long as wide and ending more or less bluntly. The genital pore is just anterior to the centre and irregularly alternating.

The excretory and nervous systems are typically tænioid in character.

The genitalia are present as a small faintly staining mass in the centre of the immature segment. In the mature segment they are fully developed; while in the gravid proglottid only the distended uterus filled with eggs and the remains of the cirrus and vagina are seen.

The *male organs*. There are twenty to twenty-four small globular testes scattered throughout the mature segment. They are not present, however, in the anterior portion nor lateral to the excretory canals. They are united by a very fine network of intercommunicating vasa efferentia which, uniting to form several larger vessels, ultimately coalesce to become the stout, elongated and convoluted vas deferens.

This joins the cirrus-sac and is continued as the cirrus to the genital pore. The cirrus-sac is elongated and pyriform in the young mature segments and reaches almost to the middle line. It does not grow, however, and in older segments it becomes more spherical and is relatively smaller.

The female organs. The ovary is typically tænioid in form—double with a uniting commissure. The halves are roughly kidney-shape in outline. Just anterior to the commissure is the spherical shell-gland. Posterior to the ovary, but some distance from the hind end of the segment lies the yolk-gland—a spherical or elongated body.

The vagina opens at the posterior portion of the genital sinus and runs as an almost straight, thick-walled tube towards the shell gland, passing between it and the ovarian commissure. From there it passes back as a very convoluted tubule as far as the yolk-gland.

The uterus is visible in mature segments as a thin-walled tube in the centre of the proglottid extending from behind the yolk-gland to anterior to the testes. It begins to fill with ova before the male organs disappear ; and in the gravid segment becomes an elongated sac almost completely filling it. The lateral diverticula so characteristic of the genus Tænia are not obvious in this genus.

Recently (1924) Brumpt and Joyeux described, under the name of *Echinococcus cruzi*, the Hydatid stage of an Echinococcus from the Agouti (*Dasyprocta agouti*) from Brazil. They were not in possession of the adults. They considered that their species differs from *Tænia ologarthra* in the following points:—

(a) the size respectively for the large and small hooks in the Hydatid is ·038 mm. and ·030 mm., whereas those of Diesing's species are ·047 and ·032 mm.

(b) the shape of the hooks differs and size dimorphism is more sharply marked in *T. oligarthra* than in *E. cruzi*.

(c) the number of hooks as given by Luhe as present in *T. oligarthra* is " upwards of 30 " while in the hydatid there are 38.

Leuckart (1886) shewed that the hooks in a Hydatid were immature and that after ingestion by the definitive host they continued their

growth. This growth however was limited to the handle and guard. The blade did not grow and was identical in both adult and larva. A comparison with Brumpt and Joyeux's figures of the Hydatid and those of the adult worm (Fig. 2, a) shews that the blade is identical in both cases and that the differences are due entirely to the extra growth of the guard and handle. Examination of hooks of adult and Hydatid of *E. granulosus* shews that the growth is greater in the case of the large hook than in the small, and that consequently the size dimorphism is much more marked in adults than in larvæ. Moreover, in a very immature form of *E. oligarthra* examined by the writer, the hooks are found to correspond very closely to those of *E. cruzi*.

Brumpt and Joyeux admit that the number of hooks is not an important point in identification owing to the readiness with which hooks are lost in the adult worms. In the specimens examined by the writer the number of hooks in this species was found to vary from 36 to 40.

The writer is therefore of the opinion that *E. cruzi* is a synonym of *E. oligarthrus*—a conclusion which is strengthened by the fact that both specimens came from the same geographical district.

Echinococcus granulosus.

As mentioned above, the state of preservation of the material of this species at the disposal of the writer is not sufficiently perfect to enable a detailed study to be made. In most cases the hooks have disappeared but drawings have been made of representative hooks from a series of specimens recovered from an English fox (Fig. 2, b). On comparison with the hooks from a number of specimens of Hydatid collected in England (from the horse) it is obvious that both belong to the same species (Fig. 2, c and d). This comparison also substantiates Leuckart's claim that the shape of the blade is permanent and that subsequent growth is confined to the handle and guard. The hooks differ from those of *E. oligarthrus* in being considerably smaller (·034 and ·030 mm. respectively). The large hook is, in proportion, much smaller than the small hook ; and size dimorphism is much less marked than in Diesing's species. In both small and large hooks, the blade is shorter and less curved ; the handle is short and narrow ; and the guard is smaller and rounder.

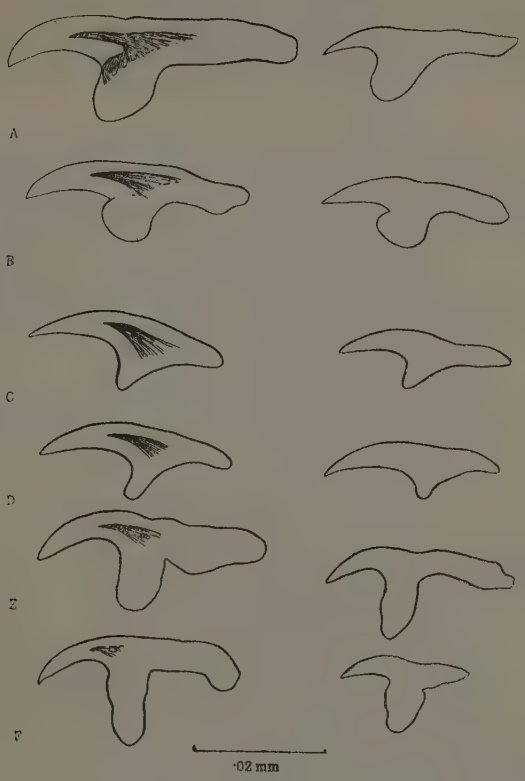


Fig. 2.—Hooks of various specimens of Echinococcus. The left-hand hook in each pair is the large hook; the right-hand hook is the small. In each case, large and small hooks were drawn from the same scolex; and all except c, d and g were drawn from specimens containing eggs.

(a) *E. oligarthrus* from *Felis yaguarundi* (S. America).
(b) *E. granulosus* from *Vulpes vulpes* (England).
(c & d) Immature hooks from Hydatid (Horse, England).
(e) Echinococcus from *Lycaon capensis* (South Africa).
(f) Echinococcus from *Canis lupus* (Macedonia).

The specimens from the fox are larger than those from dogs (reaching a length of 7 mm. as against the 5 mm. of dog forms). Both are however much larger than the South American species. The fox specimens contain two or three immature segments, but only single mature and gravid proglottides ; those from the dog have the typical three segments.

Discussion.

The size and shape of the hooks of *E. oligarthrus* and the constantly smaller size of the worm, leave no doubt as to the fact that it belongs to a different species from *E. granulosus* of Europe. It is of interest to note that so far Diesing's species has only been found in cats ; while it is well known that the common form can be induced only with great difficulty to grow in cats although it grows freely in all members of the Canidæ.

It is not possible to distinguish between the two forms on the basis of the genitalia at the present time. The details are very similar ; but more perfectly preserved material may reveal differences not at present discernible.

Echinococcus longimanubrius sp. nov.

The writer has had the opportunity of examining some specimens of an Echinococcus collected from the Cape Hunting Dog (*Lycaon capensis*). Vevers (1922) has suggested that this may represent a new species, and after an examination of the above two forms the writer agrees. The material, however, is not sufficiently well preserved to admit of a detailed examination, and in general appearance it closely resembles the ordinary species from the dog. There are, however, marked differences in the shape of the hooks.

The large hook is ·035 mm. long. The blade (Fig. 2, e) is comparatively slender and markedly curved ; where it joins the handle there is a distinct double notch. The handle is short and thickened in the centre ; its end is round. The guard is massive and cylindrical with a rounded end. The small hook is ·030 mm. long. The blade has the same characters as has the large. The handle is long and slender and is set at an angle to the blade ; its end is irregular. The guard is narrow, elongated and pointed.

ECHINOCOCCUS MINIMUS sp. nov.

This form had been collected from *Canis lupus* in Macedonia and only a few specimens were available for study. It closely resembles that found in the fox in this country, except for the shape of the hooks, which is very different from any others examined.

The large hook is ·032 mm. long. The blade is narrow and straight and joins the handle without any break in the contour. The handle is elongated, smooth, with parallel sides, and is bent at its end towards the guard. The guard is set sharply at right angles to the handle, and is very long and cylindrical with a pointed end.

The small hook is smaller than any other; it is only ·02 mm. in length. The blade is short and broad, and only slightly curved. The handle is short and conical. The guard is very massive and is irregularly conical and set at right angles to the blade and handle.

The writer is of the opinion that the two last forms differ specifically from *E. granulosus* and *E. oligarthrus*. The material, however, is scanty and future examinations of more abundant material may shew that they are really members of the type species of the genus.

The hooks however differ radically in shape and size from both known species. In the various species of the genus Taenia found in dogs, the hooks remain constant in both of these characters within any single species, and although other morphological differences exist, the appearance and size of the hooks remain the most striking and the most constant features. It seems reasonable to assume that the hooks in such a closely related genus as this is, will also within small limits remain constant in shape and size for each species. Such was found to be the case in all the examples of *E. granulosus* and of *E. oligarthrus* studied by the present writer. Accordingly it is proposed that the species from *Lycaon capensis* be called *Echinococcus longimanubrius* on account of the long handle of the small hook; and that the species from *Canis lupus* from Macedonia be called *Echinococcus minimus* because the small hook is the smallest found in this genus.

References.

Brumpt, E., and Joyeux, C., 1924.—" Un nouvel Echinocoque, Echinococcus cruzi." *Ann. de Parasit. hum. et comp.*, II., pp. 226-231.

Diesing, O., 1863.—*S.d.k. Akad.d. Wiss. Wien, math-naturw. Cl.*, vol. XLIX., Abth. 4, p.370.

Lühe, M., 1910.—" Cystotanien sudämerikanischer Feliden." *Zool. Jahr.*, supp. 12, III., pp. 687-710.

Vevers, G. M., 1922.—" On the Cestode parasites from Mammalian hosts . . ." *Proc. Zool. Soc.* London, pp. 921-928.

Journal of Helminthology, Vol. IV., *No.* 1, *March*, 1926, *pp.* 23-26.

On a New Species of Trichostrongyle worm from the Bennett's Wallaby.

By Thomas W. M. Cameron, M.A., B.Sc., Ph.D., M.R.C.V.S.

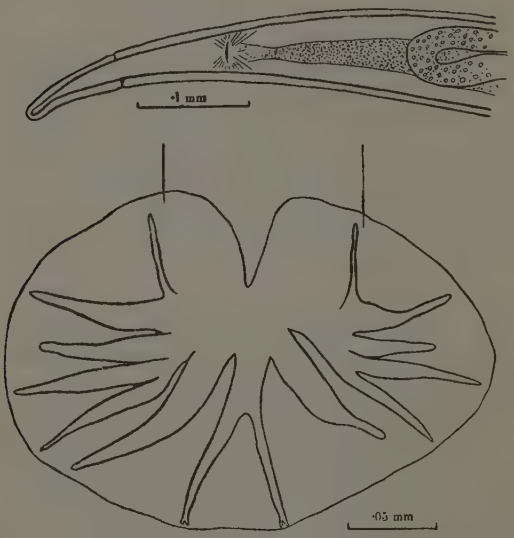
(*Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

Recently (1924) Chandler has described from the duodenum of a Bennett's Wallaby (*Macropus bennetti*), a new genus of Trichostrongylidæ. Shortly after the publication of this paper, the writer was privileged to study what appears to be a second new species from the same host. The parasites are very small forms and occur mainly in the stomach, but are also found occasionally in the first part of the small intestine. A few specimens of Chandler's species were also present.

The worms are colourless. The cuticle is very finely transversely striated. The mouth is of the simple Trichostrongyle type but possesses a small, somewhat elongated buccal cavity. No buccal teeth are present. Six papillæ surround the mouth opening. The œsophagus is about ·1 to ·15 mm. long, and is only slightly swollen at the posterior end. The nerve ring lies just anterior to the middle of the œsophagus. The *female* is about 8 to 10 mm. long and ·3 mm. thick. The tail terminates in a blunt rounded point (Fig. 1) which is occasionally swollen. Caudal papillæ are present but are very minute and, opening into small crypts, do not appear above the surface of the cuticle. The anus, which is a comparatively broad transverse slit, lies about ·2 mm. from the tip of the tail. The vulva is also a very broad transverse slit and is situated in the posterior fifth of the body. The genitalia are double. The ovejectors are of the typical muscular Trichostrongyle type. The uteri are long and almost straight tubes. The anterior uterus passes forwards towards the head, and becoming the ovarian tubule, folds back on itself just before reaching the œsophagus and almost immediately terminates. The posterior uterus passes backwards to a short distance

in front of the anus where it turns, and the ovarian tubule passing forwards, terminates some distance anterior to the vulva. Transverse coils are absent and only in the anterior portion are longitudinal folds seen.

The *male* is smaller than the female, being about 2·5 mm. long and ·25 mm. broad. With the exception of the bursa and spicules, the genital organisation is typical.



Trichostrongylus asymmetricus, sp. nov.

Fig. 1.—Posterior extremity of Female.
Fig. 2.—Bursa of male, viewed from ventral surface.

The bursa is of the general open Trichostrongyle type but exhibits a definite, although slight, asymmetry. The ventral rays are widely separated (Fig. 2), the ventro-ventral being slender with parallel sides while the latero-ventral is large and tapering. The lateral group are

fairly close together but diverge distally. The externo-lateral is blunt and short, never reaching to the margin of the bursa. The medio-lateral and posterio-lateral reach the edge of the bursa. The dorsal group consist of two stout externo-dorsal rays and a single dorsal which bifurcates about one-third of its length from the base. Each bifurcation has two terminal digitations.

The rays on the left side of the bursa (*i.e.*, the observer's left when the bursa is viewed from the ventral aspect) are stouter and longer than those on the other side, and this tends to give the bursa an asymmetrical appearance. This feature is constant in all the examples studied, although never well marked.

Trichostrongylus asymmetricus, sp. nov.

Fig. 3.—Spicules and gubernaculum.

The bursal membrane is "stippled" marginally and covered with concentric striations internally.

The spicules (Fig. 3) resemble those of the genus Trichostrongylus. They are similar, short and pointed with one long main point and two short subsidiary points. The gubernaculum is trowel-shaped and of a very simple character.

Discussion.

The only species hitherto described from Marsupials is *Austrostrongylus macropodis* Chandler, 1924. It differs from this form in many respects —especially in the shape and size of the spicules, in the disposition of the bursal rays, in the tail of the female and in the presence of a buccal tooth.

The general characters of the present species are very close to those of the genus Trichostrongylus. The small size, the widely separated ventral rays, and the shape of the spicules are those usually associated with this genus. The presence, however, of a slight but definite asymmetry of the bursa and the bursal rays separate it from all other known species in this genus. The writer is, however, of the opinion that it should for the present at least, be placed in the genus Trichostrongylus, and he proposes the name *Trichostrongylus asymmetricus* for it.

REFERENCE.

CHANDLER, A., 1924.—A New Genus of Trichostrongylid Worms from the Kangaroo. *Parasitology*, XVI., pp. 160-163.

Journal of Helminthology, Vol. IV., *No.* 1, *March*, 1926, *pp.* 27-30.

Hexatylus viviparus gen. et sp. nov., a nematode found in a diseased potato tuber.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine*).

Introduction.

Out of a large number of diseased potatoes sent by a merchant to this Institute and to the Ministry of Agriculture Pathological Laboratory, Harpenden, suspected of eelworm disease due to *Tylenchus dipsaci*, only one tuber was found by Mr. Buckhurst of the latter laboratory to contain eelworms; all the others, although presenting a naked-eye appearance extremely like that shown by tubers attacked by *T. dipsaci*, were entirely negative. This tuber was sent to the writer for his opinion on the nematodes living in it with the result that what appears to be a new genus has been found. It resembles members of the genera Tylenchus and Aphelenchus in that it has a mouth stylet with swellings at its base and might at first sight be mistaken for a representative of one of these genera, a fact which renders a description of it all the more necessary, but differs from them in several morphological details.

The most striking difference is the absence of a muscular œsophageal bulb corresponding to the first œsophageal bulb in Tylenchus or the single bulb in Aphelenchus. Another marked difference is the presence of six swellings instead of three at the base of the stylet, a character on which has been thought desirable to form the generic name Hexatylus. Lastly, the vulva is situated in a position much more posterior than in Tylenchus and Aphelenchus and the uterus possess no post-vulvar diverticulum.

So far only female specimens have been found in spite of a diligent search for males and it is of course possible that it may be a parthenogenetic species. The writer is also uncertain whether it is a true parasite

but is at present inclined to regard it as a saprophytic secondary invader in that it has only been found amongst dead plant cells and not at the edge where diseased and healthy tissues meet as happens with *T. dipsaci* in potatoes. Moreover as has already been pointed out, this was the only diseased tuber which had nematodes in it.

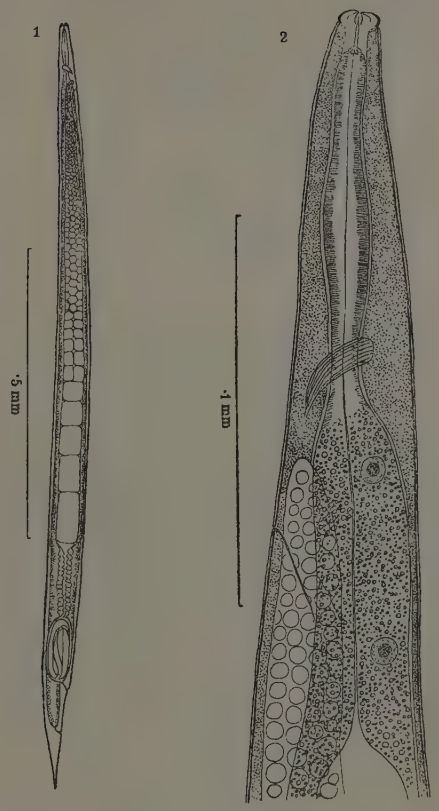
A survey of the literature has failed to reveal any published description which agrees with this worm and it is therefore placed in a new genus.

Morphology.

The principal measurements of mature worms are as follows:— total length, ·76 to ·9 mm.; greatest breadth, ·045 to ·057 mm.; buccal stylet, ·01 to ·011 mm.; nerve ring from anterior end, ·063 to ·075 mm.; excretory pore from anterior end, ·083 to ·09 mm.; vulva to tip of tail, ·083 to ·095 mm.; anus to tip of tail, ·05 to ·059 mm.

The general appearance of a mature worm is shown in Fig 1, where it can be seen that the body is comparatively stout. It tapers anteriorly and posteriorly and from the vulva onwards the narrowing is quite pronounced so as to produce a pointed tail. The cuticle is transversely striated with fine striæ. The head end is covered with cuticle which has the appearance of a flattened cap almost circular in outline. It is crossed by six ridges of cuticle arranged like the diagonals of a hexagon between which the surface is somewhat sunken.

The mouth aperture is terminal and central and leads into a short well-defined buccal cavity the sides of which are convex. The extremely fine point of the mouth stylet lies in the buccal cavity and the stem of it which is slender extends backwards and expands at its base into six swollen lobes which radiate outwards from it. From the centre of its base the stylet is connected with the lumen of the œsophagus. The latter is without muscular bulbs or swellings anterior to the nerve ring. Where this crosses, it is slightly constricted and then expands again into a region with thick walls which blend imperceptibly with the anterior end of the intestine. It is probable that this part corresponds to the second œsophageal swelling in Tylenchus and the distinct salivary glands of Aphelenchus for what appear to be large nuclei, 2 or 3 in number have been seen lying in it though with difficulty owing to the presence of refractive food bodies. After careful and pro-



Hexatylus viviparus, gen. et. sp. nov.

Fig. 1.—Entire worm under low magnification to show general features.
Fig. 2.—Anterior end under high magnification.

longed examination of this region both in fresh and preserved worms mounted in glycerine the writer can only record that he can find no special posterior demarcation of the œsophagus from the intestine. The lumen passes through the region as a narrow tube with distinct walls and then expands into the cavity of the intestine. The walls of the latter are richly supplied with refringent granules, and in most mature specimens the intestinal lumen is distinctly visible passing right back to the short rectum connecting with the anus.

The gonad is single and extends anteriorly from the vulva almost as far as the nerve ring. The vulva is a transverse slit stretching right across the ventral surface of the body. Its anterior lip is rounded and slightly protuberant. There is practically no vagina ; the uterus connecting directly with the vulva. Very frequently one large egg is to be seen lying in the posterior part of the uterus ; occasionally two may be found there. The uterine wall where not distended by an egg can be seen to be composed of small rounded cells which present a corrugated appearance in outline. No distinct receptaculum seminis is recognisable between the uterus and the ovary such as is found in *Tylenchus dipsaci* and *T. hordei*. The ovary is made up of a strand of cells, lying ventro-laterally to the intestine, which gradually decrease in size as one approaches the anterior end of the body. Each component cell has a large nucleus with central karyosome. In fully mature worms the one or two eggs in the terminal part of the uterus have been found to be in an advanced state of segmentation or to contain a fully developed embryo, and this together with the fact that no free eggs have been found with the numerous larvæ in various stages of development in teasings of the tuber material, leads the writer to the conclusion that the worm is viviparous in habit.

References.

Cobb, N. A., 1919.—Nematology, The Orders and Classes of Nemas, One Hundred New Nemas. Washington.

Man, J. G. de, 1884.—" Die frei in der reinen Erde und im süssen Wasser lebenden Nematoden der Nierderländischen Fauna." Leiden.

Stiles, C. W. and Hassall, A., 1920.—Index-Catalogue of Medical and Veterinary Zoology. Roundworms. Bull. No. 114, Hygienic Laboratory, Washington.

Journal of Helminthology, Vol. IV., *No.* 1, *March*, 1926, *pp.* 31-35.

A Preliminary Survey of the Nematode and Cestode Parasites of Sheep in North Wales, October, 1923, to September, 1924.

By W. Norman Jones, B.Sc.

(*Laboratory of Agricultural Zoology, Department of Agriculture, University College of North Wales, Bangor.*)

As a result of complaints received from farmers as to the presence of parasitic worms in sheep, an attempt was made during the period October 1923 to September 1924 (inclusive) to ascertain the distribution and relative abundance of the more usual parasitic worms inhabiting the alimentary canal of sheep in North Wales. Regular collections were made from the following centres :—Pwllheli, Carnarvon, Bangor, Conway and Llandudno, with a few records also from Colwyn Bay, Bethesda, Menai Bridge and Denbigh. All the sheep examined were slaughtered for food purposes, practically the whole being obtained from slaughter houses. Almost all had grazed for considerable periods on the farms in the district mentioned, at elevations varying from sea level to 600 feet.

The area under examination is subject to a wet climate and mild winters ; the rainfall ranging from about 32 to 50 inches for most of the area in question. The bulk of the land is under pasture, much of which is of long standing and ill-drained. During the period of investigation, persistent rainfall was the rule, with a very abundant growth of grass. In addition to the standing flocks of this area, it is customary to winter great numbers of mountain sheep on the lowlands, and very few sheep go direct to the butcher from the mountain pastures. This rendered it almost impossible to obtain clear data as to the intestinal parasites of mountain sheep.

The technique adopted was practically identical with that employed by Cameron (1) for Scotland ; the contents of the intestines, after being collected in pails, being passed through a nest of sieves (as used by Hall (2)), the worms being collected therefrom after washing, and removed to the laboratory for further examination. Although several good Municipal Abattoirs exist in North Wales, considerable difficulties were encountered in some places, as to space available, water, etc., while the number of sheep slaughtered varied very widely from week to week, rendering any really sequential examination impossible.

In all, 1,216 sheep were examined, and these came under the following classes :—(1) Ewes 51 ; (2) Wethers 557, Lambs 608. These latter can again be sub-divided into (*a*) Lambs born in 1923, of which 242 were examined ; and (*b*) Lambs born in 1924, totalling 366. It will be seen that lambs in group (*a*) ranged from 6 to 12 months old, while those in group (*b*) were from 10 weeks to 6 months old. These came from the following districts :—Pwllheli, 84 ; Carnarvon, 187 ; Bangor 10 ; Lower Conway Valley, 452 ; Llandudno, 178 ; Rhyl, 65 ; Anglesey, 226. From unknown sources, 14 = 1,216.

The numbers of sheep examined per month from October to September were respectively :—October, 11 ; November, 101 ; December, 79 ; January, 15 ; February, 119 ; March, 312 ; April, 165 ; May, 171 ; June, 48 ; July, 158 ; September, 37. It will be noted that no records were obtained in August ; this was owing to the indisposition of the writer.

The following species were obtained :—*Moniezia expansa, M. planissima, M. alba, Ascaris ovis, Monodontus trigonocephalus, Nematodirus filicollis, Ostertagia circumcincta, Hæmonchus contortus, Œsophagostomum venulosum* and *Trichuris ovis*. Intestinal parasites were obtained from 1,147 sheep out of 1,216 sheep examined, or from 96 per cent., only 69 sheep being apparently free.

CESTODA.

Moniezia expansa proved to be the most abundant species although *M. planissima* was also very common. Both occurred throughout the year, although, as pointed out by other writers, the heaviest infestations occur in lambs in May, June and July. Both species are particularly

abundant in Southern Anglesey, but occur on all the old damp, low-lying coastal pasture land throughout the area examined, all from below 200 feet. Records were, however, obtained from an elevation of 600 feet in Flintshire. Butchers and others state that these worms do not occur in sheep reared on the higher mountains, and attempts were made to check this, but without success. Few sheep go direct from these regions to the butcher, but in the few wethers examined no Moniezia were found.

In several instances *expansa* and *planissima* occurred together.

Moniezia alba proved to be relatively scarce and only occurred in young lambs during May and June. On two occasions it was taken with *expansa*. In one lamb from Anglesey proglottides totalling 80 feet were obtained (seven scolices were present) and the lamb was slaughtered in good condition. In no case was it possible to attribute poor condition to the tapeworms present. In all, 715 Moniezia individuals were counted and the apparent average number of Moniezia per host = ·6, or real average = 1·9.

831 or 68·3 per cent. of the sheep were not infected with Cestodes, but the actual number not infected was greater than this, because in some cases ten sheep were examined together, and only one or two Moniezia individuals were counted, and so these sheep have been eliminated from these figures. An instance might be given of twelve lambs examined collectively which yielded fifty individuals.

NEMATODA.

Ascaris ovis. Two specimens were obtained from lambs near Carnarvon on April 16th, 1924. These were both males, and measured 92 mm and 54 mm. respectively. These lambs were not more than ten weeks old, and were among the earliest killed at Carnarvon in 1924.

Monodontus trigonocephalus. This was one of the most abundant, and also the most generally distributed species. It was particularly numerous during the period November to May, and 195 specimens were collected from one lamb reared near Carnarvon. This species did not

occur in lambs of the 1924 group (up to September 30th, 1924). During the counts made it was noted that in this species the number of males was practically 50 per cent. of the females. They were observed in copula on sixteen occasions. The apparent average number per host = 3, but 579 sheep were infected, *i.e.*, 52·3 per cent. of the sheep were free from Monodontus, so the real average number per host = 6·3.

Nematodirus filicollis. This species is widespread, and the records show it to occur abundantly from April to November. None were taken in January, February or March. This agrees very nearly with the Scottish records of Cameron (1., p. 57). 400 individuals were counted from one lamb (19th June, 1924) reared near Rhyl.

Ostertagia circumcincta. A large number were collected during the months of March, June, July and September, more particularly June and July. 175 (or thereabouts) were obtained from each of a number of lambs from the Conway Valley (23rd July, 1924), and 63 from a lamb near Rhyl (19th June, 1924). It was most common in the lambs born in 1924, the first record obtained being 40, a lamb from near Pwllheli (13th March, 1924), and one of the earliest slaughtered in 1924, while others were obtained from wethers on the same date.

Œsophagostomum venulosum. This also is an abundant species. It was taken most commonly during November, January and February, being less frequent during the period April to July, although over 800 sheep were examined during those months. The highest total was 70 in a wether from near Carnarvon (30th January, 1924). Œsophagostomum has a wide distribution in North Wales. The number of males was again about 50 per cent. of the females. The apparent average number per host = 0·7, but the real average = 3·7 as only 233 sheep were infected with *O. venulosum* and 80·8 per cent. of the sheep were free.

Trichuris ovis. This also occurs throughout the year, but is seldom present in large numbers. The highest count made was 67 individuals in a lamb at Rhyl. This lamb was in poor condition, but another similar lamb from the same flock and on the same date yielded only six specimens. In both of these lambs there were also a few *Moniezia planissima.*

One hundred sheep examined individually gave the following percentages of infestation, and average number per host.

	Per Cent. Infestation.	*Average No. per host.*
Moniezia expansa	5	5·2
„ planissima	12	2·0
Monodontus trigonocephalus	18	27·0
Nematodirus filicollis	21	—
Ostertagia circumcincta	17	—
Œsophagostomum venulosum	25	13·4

Moniezia spp. Monodontus and Œsophagostomum occurred together in the same sheep very frequently, as also did Ostertagia and Nematodirus. Hæmonchus, when it occurred, was also associated with these two. *Moniezia expansa* and *M. alba* were twice taken in association.

Throughout the work, attention was given to the general condition of the sheep examined, but no correlation can be said to have been established between the presence of any of the parasites and marked poverty of condition in the host ; frequently the best lambs from the commercial aspect harbouring more parasites than others in less good condition.

The writer wishes to acknowledge the valuable assistance and advice of C. L. Walton, M.Sc., Ph.D., throughout the work, and to express his indebtedness to Professor R. T. Leiper, M.D., F.R.S. ; Professor Warrington Yorke, M.D. ; T. Southwell, Ph.D. ; and T. W. M. Cameron, Ph.D., M.R.C.V.S., for kind advice and assistance in identification, also the municipal official in charge of the various abattoirs visited, all of whom have been most helpful.

Literature.

1. Cameron, T. W. M., 1923, Jl. of Helminthology, vol. I., pp. 53-66.
2. Hall, M. C., 1922, Jl. Lab. & Clin. Med., II., pp. 347-353.

Journal of Helminthology, Vol. IV., *No.* 1, *March*, 1926, *pp.* 36-42.

A Further Survey of the Nematode and Cestode Parasites of Sheep, Pigs, and Cattle in North Wales, October, 1924, to September, 1925.

By W. Norman Jones, B.Sc.

(*Laboratory of Agricultural Zoology, Department of Agriculture, University College of North Wales, Bangor.*)

The Ministry of Agriculture and Fisheries awarded the writer a special research grant to continue the investigation of the helminth parasites in the alimentary tract of sheep and other ruminants in North Wales.

The area under investigation was more limited than that of the previous year, regular collections being made only at Bangor and Carnarvon. The animals slaughtered at these abattoirs are drawn chiefly from districts in Anglesey, and from a radius of about eight miles from Bangor and Carnarvon. It was proposed to restrict the number of abattoirs used for collection, and to follow up any interesting regional distribution that might show in collecting material at these central stations.

The sieves described by Hall and Cameron, and used by the writer in previous year were substituted by the sedimentation method. This latter method being similar to that used by Morgan. The contents of the small intestine were run out between the fingers into a bucket, and then transferred to a small milk can. Twelve of these milk cans were used, which were transported by means of a light wooden crate containing twelve sub-divisions. The remainder of the intestinal tract, and the abomasum were carried intact to the laboratory in a large pail. The contents of the small intestine were emptied out into wide dishes, and water was added. Then by process of sedimentation and decantation several times, the super natent liquid eventually cleared, and the worms were picked out, and put into normal saline. These were identified before fixing, or were fixed in glycerinated alcohol (equal volumes of pure glycerine and absolute alcohol) for later examination. The contents of the abomasum were abstracted by making an incision

along the whole length and washing the lobes clean with water into a dish, and the contents of the large intestine were treated similarly. This was found more easy to clean than the abomasum, and *Trichuris ovis* adhering to the walls could be removed with a forceps. The sedimentation and decantation method was again used as in the case of the small intestine. The sieves were used occasionally to separate and break up the coarser material, and were then washed into a dish to recover any parasites which might have adhered to the meshes. It is emphasized that the sieve method was only used to supplement the sedimentation and direct visual observation, which method proved more efficient for the collection of the smaller parasites, because of their tendency to become invisible on the finer meshed sieves.

During the year 160 sheep were examined, and these can be divided into classes (a) 1924 lambs = 59; (b) older sheep, ewes and wethers = 48; and (c) 1925 lambs = 53.

Five further species were recorded for the area, namely, *Cooperia oncophora*, *Cooperia curticei*, *Trichostrongylus vitrinus*, *Ostertagia trifurcata*, and *Chabertia ovina*.

These, however, were of occasional occurrence, and generally present in small numbers. Twenty sheep were free from any parasitic infestation.

Moniezia spp. *Moniezia expansa* was recorded on twelve occasions, and *M. planissima* nine times. *M. alba* appeared twice, both during June. There were 28 other Moniezia records which were not identified. The Moniezias were counted by their scolices, and it is possible that the number harboured would be slightly in excess of the figures given, due to a few of the scolices not being freed from the wall of the intestine. This part of the sheep being a by-product, it was not possible to have every case examined. None were found in small intestines which were procured and examined along their entire length. The total number of Moniezia records was 51, *i.e.*, 68·2 per cent. of the sheep were not infected with cestodes. From the period October to the end of March they were almost completely absent, only three records were made. In the preceding year there were 59 records during the same period. 188 individuals were collected, and the apparent average number of individuals per host = 1·2, and the real average = 3·7. The largest number of individuals was 43, from a lamb bred in Anglesey. The total length

of the proglottides was 125 feet. Despite this great infestation the lamb was in good condition for slaughter. The greatest length of any complete worm was 14 feet.

Monodontus trigonocephalus. This is one of the most common of the intestinal parasites, and is often the only species present, and it has a wide distribution in North Wales. No records were taken after April, as it does not occur in young lambs, and no older sheep were examined in these months; but it becomes established when lambs are from eight to nine months old. It was recorded on sixty-six occasions, *i.e.*, 58·8 per cent. of sheep being free from Monodontus. The number of worms collected was 774, and the apparent average number per host =4·8, and the real average number = 11·7. The apparent average was greater than in the previous year, when it was 3 per host. It is difficult to draw a fair comparison on the number of records, and real average number, as the examination in 1923-24 was not always individual, and therefore it was impossible to ascertain the exact number of sheep harbouring the parasite. Females were present in greater numbers (about double) than the males, whereas in 1923-24 about equal numbers were found. Copulation was not observed at all.

Cooperia oncophora and *C. curticei* were recorded, but they were only of occasional occurrence, and generally in small numbers.

Trichostrongylus vitrinus was taken a few times, and in small numbers only.

Nematodirus filicollis is one of the most abundant species, being recorded in this series on 51 occasions. It did not occur in January, February or March, but became abundant in summer months. 32 per cent. of the sheep were infected, and as the number of individuals present is usually high, detailed counts were not made; but in one case 236 individuals were present. They appear as tangled masses of pinkish threads.

Hæmonchus contortus. This is usually believed to be one of the species most harmful to sheep. It was found more commonly in this series than in the previous year, being recorded on 27 occasions, *i.e.*, 80·6 per cent. of the number of sheep examined did not harbour this parasite. It was taken most commonly in lambs of the 1924 group, but was found also in lambs of the 1925 group. In one case 73 individuals were taken

from one lamb of the 1925 group, and this animal was in good condition for slaughter.

Ostertagia circumcincta is one of the most prevalent species. It occurred in all months except April. 53 records were obtained, *i.e.*, 66·9 per cent. of sheep were not infected. It was present in varying numbers, usually high, and it tends to be more prevalent in the summer months, and is more common in spring and early summer than is Nematodirus.

Ostertagia trifurcata was recorded a few times, but *O. circumcincta* is the more important species from point of view of numbers present per host, and the number of sheep infested.

Œsophagostomum venulosum. There is a distinct fall in the percentage number of records as compared with the previous year. Only 31 records were made, *i.e.*, 80·6 per cent. of sheep did not harbour this parasite. The apparent average number of worms harboured was 1·1 ; the real average being 6·5. The greater number of records were made in the winter months, and up to March. Morgan (3) reports a tendency to increase in spring and summer months in the Aberystwyth area.

Chabertia ovina was recorded on a few occasions during the winter months.

Trichuris ovis. This species was common, and widely distributed, and could be found during all months of the year. It was recorded on 58 occasions, *i.e.*, 63·8 per cent. of the sheep were not infected. The apparent average number of worms per sheep was 3·4, and the real average therefore 9·4. It may be said that the real average is raised owing to one lamb which harboured 163 individuals. Trichuris appeared in 1925 lambs in early March.

Conclusion.

The sedimentation method is more efficient for detailed examination. The sieve method is the more rapid, as by it a large number can be examined collectively.

The age of the sheep determines largely what parasites are present. It was found that Monodontus occurred in the older sheep, and is then often the only parasite present. It occurred, however, during October in the lambs of the 1924 group, which were about six months old. With

the advent of the 1925 group of lambs Monodontus became rare. It may be said that the number of older sheep brought for slaughter decreased considerably in the spring and summer months.

Moniezia and Nematodirus were more common in the 1925 group, whilst the remaining parasites were of more common occurrence in the 1924 group. Ostertagia and Trichuris were abundant throughout. It would appear that as lambs grow up they tend to become free from many of the parasites with which they are liable to become infected in their first season.

It was expected throughout both years to find a higher degree of infestation than was actually discovered to be the case; but both years may be regarded as being somewhat abnormal with reference to parasites. During the year 1923-24 there was an abundant and luxurious growth of grass, and hence no necessity arose for the sheep to graze very closely. It is tentatively suggested that this may partially account for the supposed low degree of infestation. Furthermore, in the year 1924-25 there was a severe outbreak of liver rot, and an almost wholesale treatment with various preparations of male fern was resorted to in an endeavour to combat its ravages; many sheep not actually infected being dosed as a precautionary measure.

Presumably an apparently abnormally low degree of infestation may be explained by the fact that a majority of the sheep available for examination had been previously treated with male fern preparations.

The writer cannot perceive any direct relationship between the presence of parasites and the condition of the host for commercial purposes. It is a general opinion that Hæmonchus is the most pathogenic species. Two lambs which were in a very poor or low condition, and which therefore were suspected to harbour Hæmonchus failed to yield more than 6 and 8 specimens respectively. In one of these lambs however there was over 250 *O. circumcincta*, while the other lamb harboured only the Hæmonchus stated.

On one particular farm there were a number of low grade lambs believed to be suffering from Hæmonchus infection, yet no Hæmonchus could be found on examination. This farm is comprised mainly of good permanent pasture, and has been sheep-grazed for a considerable number of years, and probably the reason that there is a large percentage

of culls is that the land has become what is called " sheep sick," whatever this term may be. Moreover, a large flock is maintained and overstocking may be a contributory cause. It is difficult from these surveys to attribute any real harm to these worms in the numbers found, as considerable numbers of worms may be harboured and yet the animals reach the abattoirs in very good condition. On the other hand, lambs have been examined which were in very poor condition and yet harboured but few intestinal parasites. From his observations the writer would consider *Ostertagia circumcincta* the most harmful of the parasites collected by him in North Wales.

The deleterious effects of intestinal parasites may be reflected, possibly by a prolonged period of growth and retarded maturity of the host. The amount of the animal's nutriment absorbed by the Cestodes is doubtless greater than that taken by the Nematodes. The writer has however, not observed any direct and obvious signs of irritation on the walls of the stomach, which would result in retarding the progress of the maturity of the host.

The writer examined samples out of a flock of about 300 lambs which had not thriven as they should, and had been treated with Copper sulphate solutions, and in the few examined only Ostertagia and Nematodirus were found. In about three weeks there was a marked improvement in the condition of the lambs. From the data available it is difficult to determine whether the Copper sulphate functioned as a vermifuge or as a tonic.

It is tentatively suggested that variation in lambs of the same flock is due to inherited qualities and that there is a hereditary tendency in some lambs to thrive better than others, and this must not be overlooked in parasitic investigation.

Cattle. Concurrently with the investigation of the parasites of sheep in North Wales, some investigations were made in the case of cattle. The same technique was adopted as was used for sheep, but it was found much more laborious and tedious to work with cattle on account of the bulk and coarseness of the intestinal contents.

Fifteen cattle were examined with entirely negative results. No worms were found in any part of the intestinal tract. It may be said

that the cattle examined were all in a fit condition for slaughter, although it was endeavoured to select those in comparatively poor condition.

One specimen of Moniezia sp. was received from Carnarvon, and this is the only record which the writer has obtained of any parasite in cattle. It is the butchers' experience that tapeworms are of rare occurrence in cattle.

Pigs. Nineteen pigs were examined from different parts of North Wales. The procedure followed was similar to that used for sheep.

The most important and prevalent species proved to be the round worm *Ascaris lumbricoides*. It is of common occurrence, and has a general distribution in North Wales. It can readily be detected on entering the slaughter house, because of its peculiar musky odour; and can always be seen in quantity on the floor of the slaughter houses. The largest count which was obtained from one pig was 27 large Ascarids, each almost a foot in length. Nine of the pigs examined, however, contained no ascarids, nevertheless there is no doubt that they are more prevalent than these results seem to suggest. Two other species of round worms were obtained from the large intestine of two pigs from near Carnarvon—*Œsophagostomum dentatum* and *Trichuris suis*. From one of these pigs was obtained 153 individuals of *O. dentatum* and 45 of *T. suis*.

ACKNOWLEDGMENTS.

The writer wishes to acknowledge the encouraging advice and assistance of Dr. C. L. Walton, and also Professor R. T. Leiper, M.D., F.R.S., Dr. T. W. M. Cameron and Dr. Goodey. Also Mr. John Williams, Butcher, of Bangor, for valuable aid when collecting material.

LITERATURE.

CAMERON, T. W. M., 1923.—Jl. Helminthology, vol. I., pp. 53-56.
HALL, M. C., 1922.—Jl. Lab. & Clin. Med., vol. II., pp. 347-353.
MORGAN, D. O., 1924.—Jl. Helminthology, vol. II., pp. 89-94.

Journal of Helminthology, Vol. IV., *No.* 2, *May*, 1926, *pp.* 43-48.

Starlings as Distributors of "Gapes."

By E. Aneurin Lewis, M.Sc.

(*Demonstrator, University College of Wales, Aberystwyth, and Field Officer, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

In a previous article (1925), the author suggested that not sufficient importance had been attached to wild birds—and particularly to starlings—in relation to the spreading of "Gapes" among chickens.

The suggestions made in that preliminary account were based on the examination of the windpipes of rooks, thrushes, jays and starlings; and whereas *Syngamus trachealis* was collected from many wild birds it was the high percentage of infected starlings which attracted most attention. Of the 38 starlings killed during November and December 1924 about 37 per cent. were found to harbour the gapeworm.

It was thought desirable that a further investigation should be made into the case of the starlings; and with this point in view 482 starlings were obtained during the months of November and December 1925, and January and February 1926.

The birds were received in batches as follows:—November 16th, 37 (6 infected); November 17th, 40 (13 infected); November 19th, 20 (5 infected); November 23rd, 21 (6 infected); November 27th, 37 (11 infected); November 30th, 11 (5 infected); December 8th, 25 (7 infected); December 10th, 17 (9 infected); January 6th, 23 (8 infected); January 8th, 30 (9 infected); January 9th, 23 (10 infected); January 15th, 23 (12 infected); January 16th, 33 (16 infected); January 28th, 8 (5 infected); January 30th, 15 (6 infected); February 1st, 19 (8 infected); February 3rd, 46 (13 infected); February 6th, 21 (10 infected); and February 9th, 31 (10 infected).

The number of tracheæ infected out of the total number was 169, the percentage being 35 per cent.

It may be noted that in the preceding figures there appears a suggestion of a gradual increase in the infection of the comparative number of starlings, and that the maximum increase is inclined towards the time when chickens begin to be hatched.

The lungs were not examined for small immature gapeworms and although gapeworm lesions often occurred in the trachea when there was no gapeworm present, no count was made unless the actual worm was found. On four occasions very small specimens of the gapeworm (two sexes coupled) were found in the trachea near the bronchi.

Counting the number of worms in each infected individual, 1 worm was present in 118 cases, 2 worms in 37 cases, 3 worms in 9 cases, and 4 worms in 5 cases. The length of the worms (measuring the female only) varied from 11 mm. to 18 mm., the average length being 13·6 mm. (much the same length as the gapeworm usually found in the tracheæ of chickens).

In Wales the name for " gapes " among chickens varies from district to district. In north Carmarthenshire and south Cardiganshire it is recognised by the Welsh name " Clefyd-y-bîg " (the disease of the beak) ; in mid and south Carmarthenshire it is termed " Y bîg " (the beak) ; in mid and north Cardiganshire as " Clefyd-y-galw " (the disease of calling), a term derived from the fact that the noisy breathing seems as if the infected bird was calling ; and again " Rhythbryf " (gaping caused by a worm) is sometimes used, but this term has nearly disappeared from common use.

Gapes is common among chickens in Cardiganshire, Carmarthenshire and Pembrokeshire during March, April and May. It also occurs in young chickens hatched during June, July and August, but during these latter months there is a gradual decrease in the numbers of young chickens hatched, so that disease does not seem to be so prevalent as in the former months.

It is also common in young turkeys during May and June and it is known that the gapeworm is common in adult turkeys and pheasants throughout the year.

Although starlings were seen in large numbers during March around Aberystwyth, owing to unfavourable circumstances it was impossible to obtain any for examination for gapeworm. There is little doubt

that they harboured the gapeworm at that time.

It may be stated therefore that the adult starlings, turkeys and pheasants infected with *S. trachealis* act as a bridge between the period of possible infection of chickens from one year to another, and thus keep up a connected period for the propagation and distribution of *S. trachealis*.

For example, when there are no chickens for the gapeworm, starlings are generally abundant, and are infected to a high percentage ; and when the starlings are about to migrate—from our islands to central Europe—chickens appear, and may act as hosts for the gapeworm.

The starlings are migratory birds (pheasants less so), and move from district to district and from one country to another ; whereas the turkey is generally kept in certain restricted areas, starlings frequent the open fields, and often visit the poultry runs, to obtain food. In large flocks they visit the areas where the farm poultry are allowed to roam, and thus contaminate the soil with the eggs of the gapeworm from the infected starlings. Here probably lies the cause of a sudden outbreak of " gapes " among the chickens of a farmer who has never before suffered losses from the disease.

It has been ascertained that many poultry-keepers in Wales, who have never kept turkeys, and on whose land it is known that turkeys have never been kept, have suddenly suffered losses from gapes among the chickens. On one particular estate, about 12 miles north-east of Aberystwyth, no turkeys have been kept since the arrival of the present owner 10 years ago ; and it is extremely doubtful whether the previous occupier kept any turkeys on the land. Yet, cases of gapes have occurred among the chickens of the present owner. It is true that pheasants occur on the estate, and that they are infested with the gapeworm, but their ground is about a mile from the chicken runs and it is hardly possible that these birds would venture near the land where the chickens are kept, as the dogs roam about quite freely. On the other hand, it has been observed that the chicken runs are periodically changed, but always within a certain limit ; and this area is often frequented by large flocks of starlings.

It is more than probable then that the sudden outbreaks of gapes among these chickens was caused by the contamination of the soil of this area with eggs of *S. trachealis* in the tracheæ of infected starlings.

It is also known that on another estate 14 miles south east of Aberystwyth an extremely large proportion of the pheasants were killed off by gapes in the summer of 1925; and in the previous winter season starlings were so numerous on the estate that the game-keepers were compelled to carry out a campaign for the slaughtering of these birds. Turkeys were not used for nursing the young pheasants; and such losses from gapes had not occurred previously.

This suggests that the starlings caused the huge losses from gapes among the pheasants on this estate.

In 1917 Ransom found that out of 386 turkeys examined 23·6 per cent. were infested with *S. trachealis:* in 1920 he found, out of a total number of 679 turkeys examined, that 22·5 per cent. were infested.

The writer found that the percentage of starlings, from whose tracheæ mature gapeworms were collected, was 35 per cent. In his work on "The Turkey as an Important Factor in the Spread of Gapeworms," Ransom states that little is known of the importance of wild birds with regard to the spread of the gapeworm; and further states that the turkey is apparently the chief agent in the spread of gapeworms to new localities, and is apparently the principal source of infection to the soil on poultry farms where gapes is prevalent.

It is admitted that the turkey plays an important part in distributing the gapeworm, but these investigations into the importance of wild birds disclose that starlings, at least, are as important a factor as, if not a more important, and much more effective a distributor of the gapeworm than is the turkey.

No detailed investigations have been made into the extent to which pheasants, thrushes, rooks, jays and other wild birds help to distribute *S. trachealis*, but it is evident that these birds must be considered as carriers to a certain extent, at least, for the gapeworm has been collected from their tracheæ on a few occasions.

Starlings are known to migrate over very wide areas, and the data, collected regarding infestation of starlings with the gapeworm, show a high percentage; it is therefore claimed that starlings, in particular, are more important than the turkey as a means of spreading the gapeworm.

Summary.

(1) Of 482 starlings examined during the period November 1925 to February 1926, 169 were infested with the Gapeworm (*S. trachealis*) : the percentage of infected birds being 35 per cent. This confirms the previous observations when the writer found 14 infected birds out of 38.

(2) There is a suggestion of a gradual increase, towards March, in the percentage of starlings infected.

(3) The average length of the gapeworm in starlings is much the same as the average length of the gapeworm of chickens.

(4) Welsh names for gapes are given.

(5) Gapes among chickens occurs in Cardiganshire, Carmarthenshire and Pembrokeshire from March to August ; in young turkeys in May and June ; and in adult turkeys and pheasants throughout the year.

(6) Starlings, turkeys and pheasants act as a bridging, between November to March (when few or no young chickens occur for the propagation and distribution of the gapeworm.

(7) Cases are instanced of (*a*) a sudden outbreak of gapes among chickens kept in an area where no turkeys have been kept, (*b*) heavy losses among pheasants on an estate which starlings had previously frequented in very large flocks.

(8) Owing to the higher percentage of infection and its wider migrations, the starling is here considered as a more effective distributor of the disease than turkeys. Other wild birds, such as pheasants, thrushes, rooks and jays, also play a part in spreading the gapeworm and the disease this worm causes.

Acknowledgment.

I have to thank Professors R. T. Leiper and R. D. Laurie for advice and criticism during the investigation, and in preparing this paper.

References.

Dujardin, F., 1845.—" Histoire naturelle des Helminths," pp. 261-262. Paris.

Megnin, P., 1883.—" On the Gapes Disease of Gallinaceous Birds, etc.," pp. 1-22.

Ministry of Agriculture and Fisheries.—" The Nematode, or Roundworm Diseases of Poultry." Leaflet No. 58.

Lewis, E. A., 1925.—" Starlings as Distributors of Gapes." *Journ. of Helminthology*, vol. III., No. 2, pp. 81-82.

Ortlepp, R. J., 1923.—" The Life History of Syngamus trachealis (Montagu) v. Siebold, the Gape Worm of Chickens." *Journ. of Helminthology*, vol. I., pp. 119-140.

Ransom, B. H., 1916.—"Miscellaneous Investigations of Animal Parasites." Report of the Chief of the Bureau of Animal Industry, p. 64.

Ransom, B. H., 1917.—*Ibid.*, pp. 56-57.

Ransom, B. H., 1918.—*Ibid.*, p. 55.

Ransom, B. H., 1920.—" Gapeworm in Turkeys and Chickens." *Journ. of Parasitology*, vol. 6, pp. 200-201.

Ransom, B. H., 1921.—" The Turkey an Important Factor in the Spread of Gapeworms." Bulletin No. 939 of the United States Department of Agriculture, Washington, D.C.

Waite, R. H., 1920.—" Earthworms—The Important Factor in the Transmission of Gapes in Chickens." *Maryl. State Coll. of Agric., Bulletin* 234, pp. 103-118.

Walker, H. D., 1886.—" The Gape Worm of Fowls (Syngamus trachealis), the Earthworm (Lumbricus terrestris), its original Host, etc." *Bull. Buff. Soc. Nat. Sc.*, vol. v., No. 2, pp. 47-71. Buffalo.

Journal of Helminthology, Vol. IV., *No.* 2, *May*, 1926, *pp.* 49-52.

Some Remarks on the Etiology of Potato Disease in Lincolnshire.

By D. O. Morgan, B.Sc.

(*Research Assistant in the Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

During investigations on the Eelworm of Potatoes in south Lincolnshire during the summer of 1925 it was found that fields shewing great variation in the extent of damage done to the crop did not have a corresponding variation in the number of eelworm cysts present. It was often observed that patches where plants were doing well shewed as many cysts in the soil and on the roots as those areas where damage was most pronounced. This seemed to suggest that eelworms might not be the primary cause of the failure but a contributory factor.

Owing to the fact that other pests, chiefly fungoid, were invariably present in addition to eelworm it seemed that one might either attribute the failure of the crop to various pests, or to some factor or factors connected with soil fertility which inhibited the proper growth of the plants and reduced their resistance to disease. In order to obtain data which might throw some light on this latter suggestion, a number of fields reported to have failing potato crops were visited and as far as possible the history of the cropping, manuring and general cultivation was obtained. Soil samples from different parts of the fields were brought back to the Laboratory at the Kirton Agricultural Institute for the estimation of the cyst content.

The writer wishes to acknowledge his indebtedness to the Principal and Staff of the Institute at Kirton for their assistance during the investigation and for bringing to his notice all cases of failing potato crops reported in the district.

Rotation of Crops.

The practice of continuous cropping with potatoes is very common in south Lincolnshire particularly on the lighter soils suitable for growing the earlier varieties. In some forty fields examined where the crop had failed and where eelworm was abundant, it was found that potatoes had been grown almost continuously for many years and often followed in the same year by a cruciferous crop. In all cases the damage was found on early and second early varieties; late varieties, although cysts were often found on the roots, did not appear to suffer. It was noted however that late varieties were rarely grown *continuously* on the same fields but formed one of the crops in the three-course rotation which is practised in the district. In the writer's opinion this use of late varieties in a rotation as contrasted with the continuous cropping of earlies and second earlies may account for the fact that lates always seem to give a good yield, in spite of abundance of cysts on their roots, whereas earlies and second earlies give poor yields.

Manuring.

Some references might be made to the method of manuring for potatoes as a possible factor for the failure of the crop in the district.

Under a system of farming which might be termed "Commercial Horticulture" very little farm stock is kept and consequently the amount of dung available for manuring is negligible. It is generally held that farmyard manure should form a considerable portion of the manure used for potatoes and this should particularly be the case where soils are low in organic matter as we find in the sandy loams of south Lincolnshire. Since this form of manure is not available, one would expect to find green manuring extensively practised. This however is by no means common.

"Artificials" are heavily used by potato growers and it was quite the common practice in recent years to apply dressings of a ton and over of a compound manure to the acre. This usually contained Sulphate of Ammonia and Superphosphate with little or no Potash. The lack of potash seems especially worthy of note when one considers that a light soil contains very little of that element and that a crop like potatoes requires good supplies of potash. A farmer in a district

where the failure of the potatoes was very common, stated that he invariably used nothing but Sulphate of Ammonia and Superphosphate. It is interesting to note that the earlier workers on the eelworm of sugar beet attributed that failure of that crop to a deficiency of potash in the soil. While the writer in his experiments has not obtained any appreciable results from the use of heavy dressings of potash, it seems probable that the lack of this form of manure is at least one of the causes of the failure of the potato crop.

The question of liming might also be referred to, having due regard to the fact that potatoes grow well in soils deficient in lime, and to the influence of the latter on the prevalence of scab. The analyses of soils at Kirton Agricultural Institute have shown that about 40 per cent. of the fields have a "lime requirement." While this alone may not be a serious matter in a potato growing district, the deficiency may influence the general fertility, the health of the plant, and the value of the artificials used. The addition of lime in some experiments carried out by the writer (see Jl. of Helm., vol. III., No. 5) shewed no improvement in the crop. It was noted however that there was an entire absence of eelworm cysts on a small portion of a field which had a fair percentage of lime present while the remainder was heavily infested and shewed a considerable lime requirement.

Influence of Rotation and Manuring.

The foregoing conclusions on continuous cropping and system of manuring are somewhat supported by observations on fields where the deficiencies mentioned were partly repaired. A field which had been continuously cropped with potatoes for some 15 years shewed large patches where the crop had almost entirely failed during 1924. A dressing of four tons of sprats per acre was applied in the Autumn and the field was again planted with early potatoes in 1925. Observations on this crop during its growth did not indicate any damage as far as the general appearance of the field went, and although the roots were quite heavily infested with eelworm cysts, the yield was much better than in the previous year.

A similar field with a past history of failing crops had cabbage ploughed in on one portion and sprats on another in the Autumn of 1924. In this instance bad patches were clearly marked in the potato

crop of the following year but the yield was remarkably good and a decided improvement on the previous year. Seasonal differences may have accounted for the better yield in these cases; it was however noticed that other fields in the district were quite as badly damaged in 1925 as they were in 1924.

The effect of rotation seemed to be indicated in another field where the writer was able to get details of past crops and also make full observations on the potato crop in 1925. In this instance potatoes had been so complete a failure in 1920 that the field had to be put down under other crops in the following sequence :—Oats, Wheat, Seeds, Seeds. The seeds in 1924 were ploughed in and the field also received a light dressing of dung. Eelworm cysts were found to be extremely abundant in the soil and it seemed that the failure in 1920 might be attributed to this pest.

Eclipse were planted in 1925 and early in the season the crop looked promising. Later there was a short period when the plants seemed to be making no headway. The roots were smothered with cysts and *Rhizoctonia solani* was also equally common, the latter being referred to as " very severe " by a mycologist who examined the plants in the field. Later the crop made good growth and when lifted in August gave an average yield of eight tons of " ware " potatoes per acre. A few rows of *Tinwald Perfection* adjoining the *Eclipse* gave somewhat better results. This yield is a remarkably good one from a second early variety on a badly infested field and seemed to shew the value of a short rotation in ensuring a good crop. It would also appear that the value of such a rotation did not depend on an appreciable reduction in the number of cysts during the absence of the host plant, as this was certainly the worst case of eelworm infestation examined in the district. While it is not suggested that the eelworm had no influence on the yield in this instance the success of the crop in spite of the pest lends support to the view that the problem is primarily one of obtaining more suitable conditions for plant growth by more judicious manuring and a system of rotation. Although the frequent cropping of infected fields with potatoes very soon results in a large cyst content, the writer is not convinced that the number present bears any relation to the degree of damage done to the crop. How one is to interpret the effect of continuous cropping is difficult to answer.

Journal of Helminthology, Vol. IV., *No.* 2, *May*, 1926, *pp.* 53-60.

On the Life History of the Lungworm, *Synthetocaulus abstrusus*, hitherto confused with that of *Ollulanus tricuspis* in Cats.

By Thomas W. M. Cameron, M.A., B.Sc., Ph.D., M.R.C.V.S.

(*Assistant in the Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

In 1865, Leuckart described from the stomach of the domestic cat, a minute bursate worm which he named *Ollulanus tricuspis*. His original description was somewhat scanty, but it was amplified in later years (1867-1876). As this work is scattered throughout many pages, the following is a brief resumé of Leuckart's theory.

He was struck by the fact, that being viviparous in habit, it resembled *Trichinella spiralis*, but recognised that several fundamental differences existed between them. Ollulanus never produced more than three larvae at a time from relatively large eggs, about 60μ to 120μ long; whereas Trichinella produced numerous larvae with small eggs about 35μ long. He found what he believed were the free larvae of Ollulanus, which measured about 320μ long by 15μ wide, had a truncated oral extremity and a short tail which ended in a short S-shaped tip (Fig. 1, a). The oesophagus was between one third and one half of the total length of the intestine; several transparent vesicles could be seen at its club-shaped posterior extremity. Although there are seldom more than three embryos inside the female, larvæ were usually found in large numbers throughout the whole intestinal tract of the host, as well as encysted (in cysts ·15 to ·2 mm. in diameter) on various internal organs. The cyst wall had a connective tissue-like structure of such thickness that it might be three or four times the diameter of the enclosed space. This space was either filled by the tightly wound coils of the worm or with a clear liquid. If present in large numbers in the lungs, these capsules caused the appearance of miliary tuberculosis

—especially when surrounded by an area of hepatisation. They might even be the focus of a widespread inflammation which in one of his cases at least, led to the death of the cat. Embryos were not found in the blood or muscles, but in heavy infections were free in the bronchi.

The encysted embryos, unlike Trichinella, did not continue their development, but gradually degenerated, and finally came to resemble an egg. Leuckart fed these embryos to a mouse and six weeks later found hundreds of them encapsuled in the muscles—these forms being intermediate in form between the free embryo and the fully developed Ollulanus. The capsules—about ·3 mm.—were not only found in the rump muscles and œsophageal wall, but also in the heart and in the loose connective tissue of the neck. They superficially resembled Trichinella, but when examined microsocopically, they were found to lack the inner shell so characteristic of the trichinella capsule. The wall was a simple connective tissue one, surrounded by nodules and containing numerous granules which were moved about by the movements of the larva (Fig. 1, *b*). The enclosed worm had increased in size and was about ·8 mm. long by ·04 mm. broad. The blunt end showed a retracted chitinous disc which, from analogy, might be taken as the first indications of the future mouth. The cuticle was thicker and ringed. Round the lip-like projections were a few papilla-like outgrowths. The tail was simple and pointed. The intestine had not changed except that the pharynx was comparatively shorter and showed a muscular structure. The intestine was brown and towards its middle, the slightly enlarged bean-shaped genital primordia could be seen.

He was unable to decide whether these stages had reached maturity, but going by the results of an experiment, he considered that further development in the mouse was possible. An infected mouse was fed to a cat and eight days later the worms were recovered from it unchanged, but in the caecum and rectum, not the stomach. As only a few were found, it was believed that they were en route for the exterior and Leuckart considered that all the conditions necessary for further development were not present.

Only one other experiment is recorded. Two young rats were fed with the stomach wall of a cat infected with Ollulanus larvæ, and after

twenty-four hours and three days, respectively, the majority of the introduced worms were recovered alive and undamaged in the stomach.

Cobbold in this country was also studying the parasite although he preferred to use the name Olulanus instead of the more correct Ollulanus.

In discussing the probable life histories of the lung worms in general, Cobbold (1875) drew attention to Leuckart's belief that "all these strongyloides required a change of hosts before they can take up their final abode in the sexually mature state. This he infers especially because their respective embryos display characters very similar to those exhibited by Olulanus."

He considered the question fully in a paper read before the Quekett Microscopical Club in 1885. He stated that his first acquaintance with the larvæ of Olulanus was made about 35 years previously when a young cat in his room suddenly rushed about and finally fell dead, asphyxiated. While yet warm, it was dissected and its lungs were found to be swarming with nematode larvæ. Drawings were made but no specimens were preserved, and it is not stated whether any adults were seen in the stomach.

He then briefly recapitulated Leuckart's work on the subject and described a case which he had seen with Mr. Gay, a surgeon, in which a family, together with their donkey and cat, had suffered from a condition which was suspected to be Trichinosis. On investigation, no evidence of this was found, but the lungs of the exhumed cat were found to be swarming with "Nematoids." Cobbold considered that this was a case of "Olulanosis" and states that "Whatever interpretation be put upon the human outbreak, the coincidence of the occurrence in man of an affection symptomatic of trichinosis, found in association with a trichinoid affection in an animal which proved olulaniasis, was both curious and instructive."

No subsequent work on the subject seems to have been done, excepting some negative experimental attempts at infection by Galli-Valerio in 1921.

Meanwhile in 1890, Müller described from the cat in Germany a new form of lung-worm which he called *Strongylus pusillus*. This name was afterwards found to be a homonym of Rudolphi's *Strongylus pusillus*, and Railliet (1898) proposed the new specific name of *S. abstrusus* instead.

Later (1907) Railliet and Henry transferred this species to the genus Synthetocaulus.

Müller described this species as follows :—

This very small species is found in the pulmonary tissue and not in the trachea and bronchi, the muco-serous contents of which contain only eggs and embryos.

The body is filiform and thin, the female being coloured a darker brown than the male.

The pigmentation which is not so dark at the head as at the tail increases to moderately large granules lying in the intestinal wall which are finer in the neighbourhood of the œsophagus than at the anus.

The mouth is unarmed and no papillæ were noticed. The œsophagus is proportionally short and slender with swellings at the base. The cuticle is very thin except at the head. The *Female* is about 9·6 to 9·9 ''' long and ·05 to ·1 ''' broad. The ovaries are regular tubes and the junction of the uteri with the vagina cannot be easily recognised. The vagina is about ·19 ''' long and is thick. The translucent thin-shelled eggs lie in it singly. The vulva is ·07 ''' in front of the pointed tail, which is contrasted from the body tissue by a little prominence. The anus lies behind it and in its wall are two little chitinous " hooks " which project towards the ventral side. The eggs are small (·05 to ·07''') and have a very thin shell. They are found in all stages of development in the fluid from a resected piece of lung. The embryos which are ·27 to ·35 ''' long by ·017 to ·023 ''' thick, are characterised by an S-shaped tail. (Fig. 2, *f* and *g*.)

The *Male* is smaller and clearer than the female and is 4·9''' long and ·05 to ·08''' broad. The testes show no abnormal characters. The bursa (Fig. 2, *e*) is thin, short and ·017''' in length. The free border, seen laterally, is ·05''' long. The rays are short and stout and resemble those of Strongylus in type. The dorsal rays are short and thick with distinct notches at the free ends. The lateral are somewhat more slender. The median rays are small and separate from each other on a short common base. The spicules are long and thin and spatulate. Their

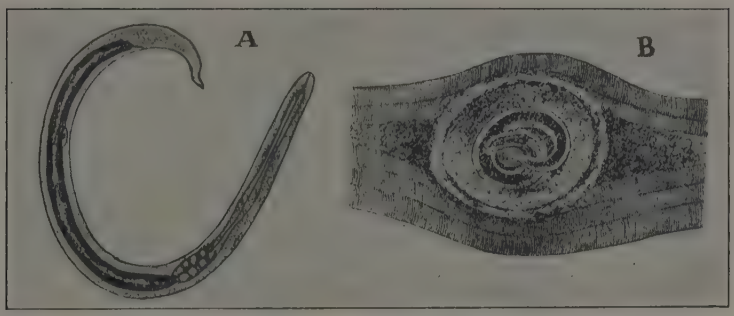


Fig. 1.—(a) Embryo of *Ollulanus tricuspis* (after Leuckart, 1867).
(b) Encapsulated embryo in muscles of mouse (after Leuckart, 1867).

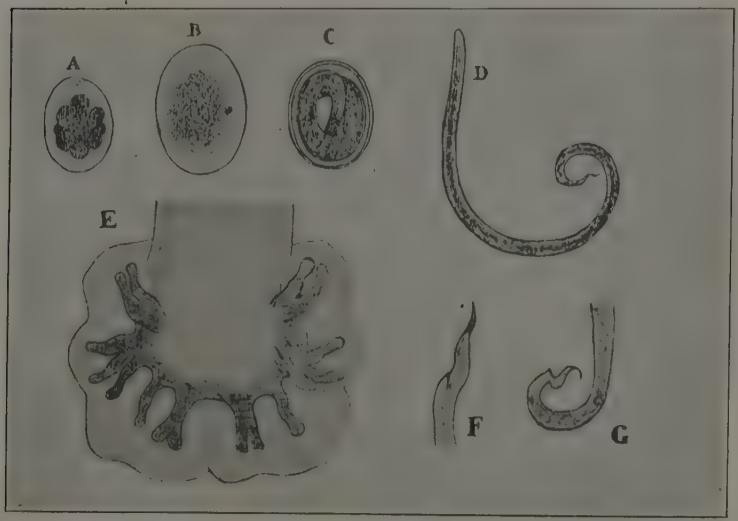


Fig. 2.—(a), (b), and (c), Eggs of *Strongylus pusillus* (after Railliet). (d) Free embryo (after Railliet). (e) Bursa of *Strongylus pusillus* (after Müller). (f) and (g) Posterior ends of embryos (after Müller).

length varies from ·01 to ·013 ‴ The figure shows the dorsum of a male with small spicules. These have a strong border which on the dorsal aspect is usually studded with fine chitinous dots and ends bluntly. This border surrounds the tail and passes further on for a short distance on the ventral margin of the spicules. Between the spicules are two short chitinous rods.

Railliet (1895) gives a brief description of this species, but as he had not seen the adults, it is presumably taken from Müller's previous paper. Railliet saw the eggs and larvæ and although his description of the larvæ agrees with that of Muller, his measurements are different. The eggs were found to be ·060 to ·085 mm. long by ·055 to ·080 mm. broad, while the larvæ were ·370 to ·459 mm. long by ·016 to ·018 mm. broad with an undulating appendix at the slender caudal end. (Fig. B, *a-d.*)

Müller's measurements are given in lines (‴), and there is some difficulty in understanding his exact meaning. A line is $\frac{1}{12}$ of an inch (*Zoll*), and is therefore approximately equal to two millimeters. Railliet in describing this species, quotes Müller's figures, but gives them as millimeters. If this is the correct interpretation to be put upon Müller's " line " then the larvæ as seen by Railliet are almost the same size as those seen by Müller: if, however, his " line " is $\frac{1}{12}$ of an inch, they are almost half the size of Müller's larvæ. Assuming that a line is approximately equal to two mm., then the size of the eggs and larvæ as given by these workers are given below. Railliet does not actually give the figures in the centre column, but they are calculated on the basis used by him for Müller's other figures.

	Müller	*Müller after Railliet*	*Railliet*
Length of egg ...	... ·1-·14 mm.	·05-·07 mm.	·060-·085 mm.
Breadth	... ·1-·12 mm.	·05-·06 mm.	·055-·080 mm.
Length of embryo	... ·54-·7 mm.	·27-·35 mm.	·370-·450 mm.
Breadth	...·034-·046 mm.	·017-·023 mm.	·016-·018 mm.

Actually Railliet's own measurements of the larvæ are almost exactly the same as in those studied by the writer. Müller so far has been the only observer fortunate enough to obtain males and females. Railliet

and Neumann have obtained portions while the latter has also found a single male and an immature female.

Both of these parasites have been mentioned in the literature since the dates of their discovery, but nothing has been added to our knowledge of their morphology and life history.

In 1921, Dr. Hesse in Edinburgh discovered some examples of *Ollulanus tricuspis* in the stomach of a cat which he had been examining: and subsequently the writer found them in other cats. Their presence was brought to the attention of Professor Leiper, who suggested to me that attempts should be made to repeat Leuckart's experiments on the life cycle of the parasite.

Search was accordingly made through numerous cats. The material collected fell into three groups: those in which mature Ollulanus were found in the stomach without any larvæ elsewhere, those in which, in addition to mature Ollulanus, numerous larvæ were found in the intestinal and respiratory tracts, and those in which larvæ alone were found. Further examination of the larvæ however made it clear that they were not the larvæ of Ollulanus, but those of some other parasite, and from the shape of the tail, this other parasite was probably a lung worm belonging to the genus Synthetocaulus. These free larvæ were identical with those described by Müller (Fig. 2, *f* and *g*), and by Railliet (Fig. 2, *d*), for *Synthetocaulus abstrusus*. Moreover, the embryos of Ollulanus when dissected out from the female were totally different from those free forms. Experimental work was undertaken and although results are not yet complete, it is sufficiently far advanced to show that Leuckart had confused two separate parasites in his work on Ollulanus. These results indicate that while the adults of *Ollulanus tricuspis* were undoubtedly seen by Leuckart (there is no evidence that Cobbold ever saw them) the larvæ seen by both Leuckart and Cobbold were those of *Synthetocaulus abstrusus* and that the stages seen in the mouse were those of the latter species also. In other words, the life cycle of *Ollulanus tricuspus* as described by Leuckart, does not apply to that species at all, but to *Synthetocaulus abstrusus*. The larvæ of this species are carried to the exterior in the droppings, ingested by mice, continue their development and become encysted in these animals, and ultimately, when the rodent is eaten by a cat, develop into the adult lung worms.

The experiments on which the writer bases this statement have occupied several years and will form the subject of a separate paper.

The life cycle of Ollulanus is still unknown, but experiments are in progress by means of which it is hoped to clear up the whole matter.

The geographical distribution of *Ollulanus tricuspis* is not fully known. It was found in Germany by Leuckart and in Switzerland by Galli-Valerio. Cobbold also reported it from Britain, but there is no clear evidence that he ever actually saw this species, and possibly his record should apply to *Synthetocaulus abstrusus*. Hall records its presence from Washington, D.C., but it is not stated whether he is referring to the adult in the cat or the larva in the mouse. In the latter case, his record would, of course, refer to Müller's species. Neumann reports that, although he searched very carefully for Ollulanus in France, he was unable to find it.

The distribution of *Synthetocaulus abstrusus* is also incompletely known. It has been reported from Germany (Müller), France (Railliet and Neumann) and from Northern Italy and Switzerland (Galli-Valerio). It has not previously been recorded from Britain, but there is little doubt that the forms seen by Cobbold and Stirling should be referred to this species.

The writer has to acknowledge the assistance of Mr. P. L. le Roux in the dissection and Mr. A. K. Cameron in the collection of material. Laboratory accommodation in Edinburgh was generously placed at his disposal by Professor J. H. Ashworth in the Zoological Department of the University. The remainder of the work was done in this Department under the direction, and with the encouragement of Professor R. T. Leiper. The writer also wishes to express his thanks to his colleagues Drs. T. Goodey and R. J. Ortlepp for material assistance in dissections and searching through teased material and for much helpful criticism.

References.

Cameron, T. W. M., 1923.—"On the morphology of *Ollulanus tricuspis* Leuckart, 1865, a Nematode Parasite of the Cat." *J. Helminthology*, I., pp. 157-160.

Cobbold, T. S., 1873.—"The Internal Parasites of our Domesticated Animals, etc." p. 125.

Cobbold, T. S., 1875.—" On lung parasites, more especially in relation to Olulanosis of Cats." *Veterinarian*, lviii, pp. 526-532.

Galli-Valerio, B., 1921.—" Parasitologische Untersuchungen und Beiträge zur parasitologischen Technik." *Centralbl. f. Bakt., etc.*, I. Abt. Orig. Bd. lxxxvi., pp. 346-352.

Hall, M. C., 1916.—" Nematode Parasites of Mammals of the Orders Rodentia, etc." *Proc. U.S. Nat. Museum*, vol. l., pp. 177-178.

Leuckart, R., 1865.—" Bericht über die wissenschaftlichen Leistungen in der nat. der niederen Thiere." *Arch. fur. Nat.*, xiii., p. 227.

Leuckart, R., 1867-1876.—" Die Menschlichen Parasiten." Bd. ii., pp. 87-88, 102-106, 122.

Neumann, L. G., 1914.—" Parasites et Maladies Parasitaires du Chien et du Chat," pp. 270-272.

Müller, A., 1890.—" Helminthologische Mittheilungen." *Deutsche Zeits.f. Thiermedicin*, xvii., pp. 58-70.

Railliet, A., 1895.—" Traité de Zoologie Médicale et Agricole," pp. 436-437.

Railliet, A., 1898.—" Rectification de la Nomenclature d'après les travaux récents." *Rec. de méd. vét.*, vol. lxxiv., pp. 173.

Railliet, A. and Henry, A., 1907.—" Sur les variations des strongyles de l'appariel respiratoire des mammiferes." *Compt. rend. Soc. de Biol.* vol. lxiii. p. 753.

Journal of Helminthology, Vol. IV., *No.* 2, *May*, 1926, *pp.* 61-68.

On the Morphology of the adults and the free living larvæ of *Dictyocaulus arnfieldi*, the Lung-worm of Equines.

By Thomas W. M. Cameron, M.A., B.Sc., Ph.D., M.R.C.V.S.

(*Assistant in the Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

This parasite was first reported by Cobbold in 1882 from a donkey and described by him two years later under the name of *Strongylus arnfieldi*. Previously, worms from the lungs of equines had been reported by Diesing and by Gurlt, but these had been confused with the species found in bovines. This species was later identified in the horse by Railliet. It was placed in its present genus by Railliet and Henry in 1907.

The morphology of this parasite has never been completely described while the life history has been almost entirely uninvestigated. The writer was fortunate in receiving some live material from the lungs of a donkey killed in London and the present account of the anatomy of the adults and of the free-living larval stages is based upon this.

Morphology of the Adult Worms.

The cephalic end is simple with a functional mouth cavity only, surrounded by six flat papillæ. The cuticle is finely striated transversely and longitudinal striations are also evident. Cervical papillæ are absent. The œsophagus is bluntly club-shaped and in the female is about ·75 mm. long by ·15 mm. broad. The nerve ring is situated about the middle of the œsophagus and the excretory pore is just posterior to it.

The *female* is about 43 to 60 mm. long and ·4 mm. broad. The genitalia are double and are very similar in type to those in other members of this genus. The vulva is situated just anterior to the middle of the body. The anus is a small transverse slit about ·4 mm. from the bluntly

pointed, tapering end. A pair of very small caudal papillæ are present but they do not project through the cuticle. (Fig. A, 2.)

The *male* is shorter than the female, being only 25-35 mm. long and about ·25 mm. broad. The genital system is single and similar to the other members of this genus. The bursal rays (Fig. A, 3 and 4) are bluntly pointed and are sharply divided into ventral, lateral, and dorsal groups. The Ventral rays are small and joined together for the greater part of their length. The Externo-lateral ray is separate from, but usually close to, the other lateral rays. The Medio-lateral and the Dorso-lateral rays are fused for the greater part of their length. The Externo-dorsal rays are long and, comparatively slender in the centre, terminate in a swollen blunt portion. The stout Dorsal ray bifurcates about half-way down its main stem but the bifurcations always remain single. The spicules are large, being about ·25 mm. long, and have a honey-comb structure. They are club-shaped and closely applied to each other. The accessory piece is small—·05 mm. long—and almost rudimentary. There are no prebursal papillæ.

The Free-Living Larvæ.

As the amount of material was limited, cultures were made by teasing up mature females in water and removing the debris by sieving. The eggs were placed in ordinary tap water in Petri dishes and incubated at 20° C.

The *Egg* is thin shelled, almost round and is about ·090 mm. in its longer diameter. It contains a living embryo when deposited. This larva very quickly hatches—in a few hours after leaving the female—giving rise to the *first-stage larva* (Fig. B, 1). This larva is about ·43 mm. long and ·015 mm. thick at the thickest part about its middle. It is accordingly about midway in length between the larvæ of *D. filaria* and *D. vivaparus*, but more slender than either. The cuticle is finely transversely striated. At the anterior end is a small cephalic vesicle. This does not seem to be the same structure as has been observed by Daubney in the case of *D. filaria*. In that species the cephalic " button " is a thickening of the cuticle, while in this it seems to be the precocious beginning of the first moult. It is present, however, in the newly-hatched larva. In addition, a similar caudal vesicle is seen. The tail differs somewhat

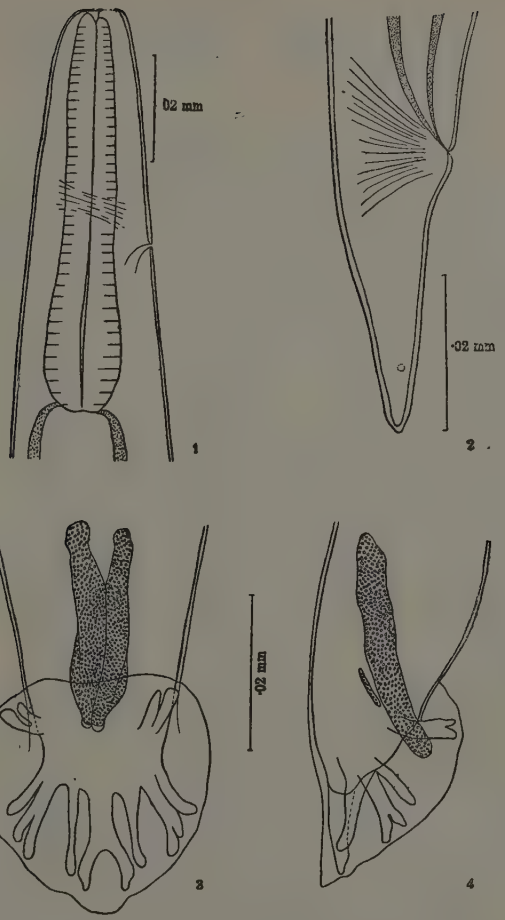


Fig. A., Morphology of *Dictyocaulus arnfieldi*.

1.—Head of female. 2.—Posterior end of female. 3.—Bursa of male, ventral aspect. 4.—Bursa, lateral aspect.

in shape from that in the corresponding stage in the other species (as described by Daubney, 1920). In *D. filaria*, the first stage larva has a blunt tail, while *D. viviparus* has a tapering tail with a fairly sharp point. In *D. arnfieldi*, however, it is spear-shaped, the spear-head forming the caudal vesicle.

Daubney was unable to see the œsophagus clearly in either of the first two stages of the other two species, but in this species it can be easily seen under the 1/12″ objective as a thin-walled tube about ·11 mm. long with a central and posterior swelling. A functional mouth is not present although thin strands are seen leading from the anterior end of the œsophagus to the posterior side of the cephalic "vesicle." Owing to granules in and around the intestine, it was not possible to ascertain the number of intestinal cells present. The rectum is seen as a thin cuticular tube leading to an anus which is probably not functional in this stage. The nerve mass is present surrounding the thin part of the œsophagus between the two swellings. The genital primordium is also seen as a cell mass, about the centre of the intestine. It does not appear to alter during any of the three free-living larval stages.

The larva completes its moult as a rule within twenty-four hours of hatching, but it does not escape from the old cuticle until after the second moult has been completed.

The *second stage larva* (Fig. B, 2) has the same general appearance as the first, the only important differences being in the shape of the tail. The anus is situated rather further from the tip of the tail which is now somewhat slender and bluntly pointed. It is not spear-shaped (as are *D. filaria* and *D. viviparus*). Neither cadual nor cephalic vesicles can now be seen ; and as there is no trace of the caudal button of the other two species attached to the cast cuticle, it is probable that this does not exist in this species. The second stage is necessarily somewhat smaller than the first as it still remains inside the old cuticle.

This stage lasts for about twelve to twenty-four hours when the larva completes its second moult—as a rule without having yet escaped from the first cuticle ; the larva is therefore now normally seen within a double cuticle (Fig. B, 3).

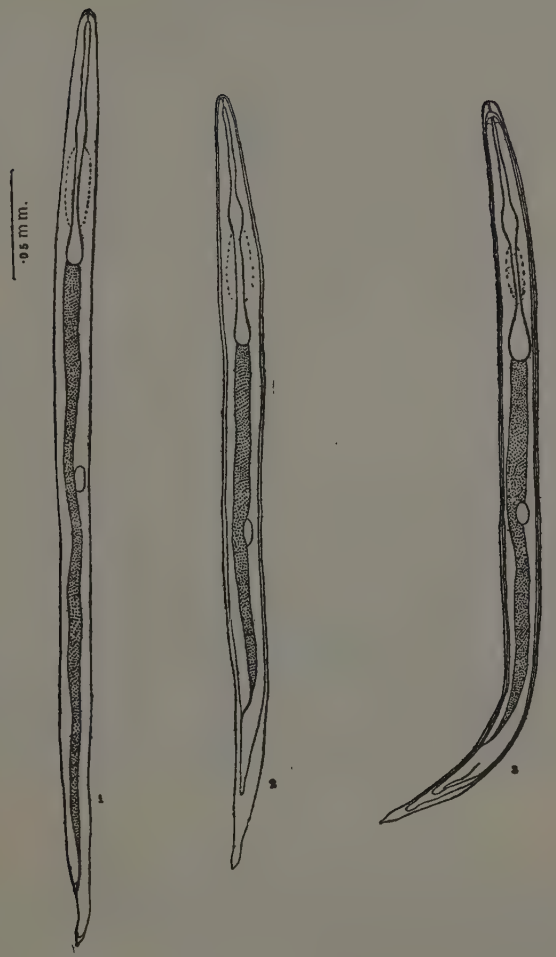


Fig. B., Morphology of the free-living larvæ of *D. arnfieldi*.
1.—1st stage larva. 2.—2nd stage larva. 3.—3rd stage larva before final ecdysis.

The internal appearance of the *third stage larva* does not materially differ from the previous stages. The œsophagus is now more obvious and the swellings are larger. The anterior portion is surrounded by a vesicle which has a totally different appearance from that seen in the first stage. In that the vesicle seems to be the commencement of the moult ; in this the lumen of the œsophagus can be traced through the vesicle. The mouth is surrounded by a group of (six ?) small flat papillæ. The tail is similar to the second stage larva but is somewhat less slender. It is bluntly pointed—not sharp as in the case of the other two species.

At a variable period after reaching this stage, the larva generally escapes from its first cast cuticle ; but in no case was a larva seen to lose the cuticle of the second stage.

The larval stages of the three species of Dictyocaulus closely resemble each other, but certain well defined differences exist between them.

Species.	*D. filaria.*	*D. viviparus.*	*D. arnfieldi.*
Host	Sheep	Cattle	Equines.
Length of 1st stage larva	·55-·585 mm.	·3-·36 mm. ...	·43 mm.
Thickness ...	·025 mm.	·024 mm. ...	·015 mm.
Tail of 1st stage stage	Bluntly tapering ...	Moderately long and fairly sharp	Spear-shaped.
Tail of 2nd stage	Slender and spear-shaped	Spear-shaped ...	Slender and blunt.
Tail of 3rd stage	Slender, elongated tail, less sharp than previous stage		Less slender.

The measurements of *D. filaria* and *D. viviparus* are taken from Daubney (1920).

Biology of the Infective Larva.

Owing to the scarcity of material, only a limited amount of experimental work could be done with the larva.

Temperature. Larvæ were found to resist cold to a considerable extent and were able to survive two days' exposure to a temperature just above freezing point.

Desiccation. Larvæ in pure water free from debris, were allowed to dry on a glass slide over night. The slide was moistened in the morning but none revived. If the larvæ were mixed with charcoal before being dried, a small percentage was found to be alive twenty-four hours later. It is probable that the charcoal prevented complete drying, however, and that had the experiment been continued longer, none would have survived. The laboratory conditions are, of course, much more severe than those generally found in nature.

Skin Penetration. Using Goodey's technique of a cork " raft " floating in warm saline and with the central aperture covered with the abdominal skin of a young rat, it was found that the larvæ did not penetrate the skin. Their movements in this experiment were quite different from those shown by Hookworm larvæ and other forms which are skin-penetrators under similar circumstances.

Migration. Larvæ did not appear to leave the water cultures at all, but as this is normally the case with such active climbers as the Hookworm larvæ, no conclusion can be drawn as to their geotaxis.

The life of the larvæ in culture was extremely short and it was found impossible to keep them alive for more than a fortnight. Railliet also found that larvæ only remained alive for a very short period when cultured in pure water ; in his experiments the period was only eight days.

The larvæ of *Dictyocaulus arnfieldi* behave in general in a very similar manner to those of *D. filaria* and *D. viviparus* so far as the properties of this species are known. Its short life, however, is a still unexplained phenomenon which seems to differ from the other species.

No attempts were made, owing to the small amount of material available, to infect any experimental animals.

Distribution.

The distribution of this parasite is probably world-wide. It has already been recorded from Europe, North and South America and Australia, but, as it frequently causes no symptoms in the affected animals, its presence is easily overlooked. It appears to be commoner in the donkey than in the horse—old or young being attacked more readily than mature animals. In the present case, the parasites were found in an aged donkey which was in excellent condition. It was stabled along

with several horses, but there was no trace of the parasite in any of these, although two were carefully examined post-mortem. Both horses and donkey were heavily infected with Sclerostomes also, but some of the species found in the donkey were not present in the horses. Looss (1902) and le Roux (1924) have observed similar phenomona in these animals, and these facts suggest that although certain nematodes may be found throughout a genus of host animals, some at least may have specific preferences within this genus. Looss believed that this might be due to different methods of feeding, but in the present case, there seemed to be no difference in treatment between the horses and the donkey.

References.

Cobbold, T. S., 1886.—" Description of Strongylus Arnfieldi (Cobb.) . . ." *J. Linn. Soc., Zool.*, xix., pp. 284-293.

Daubney, R., 1920.—" The Life Histories of Dictyocaulus filaria (Rud.) and Dictyocaulus viviparus (Bloch)." *J. Comp. Path and Ther.*, xxxiii., pp. 225-266.

Hall, M.C., 1922.—" Lungworms of Domestic Animals." *Cornell Veterinarian*, xii., pp. 131-157.

le Roux, P. L., 1924.—" Helminths collected from Equines in Edinburgh and in London." *J. Helminthology*, II., pp. 111-134.

Looss, A., 1902.—" The Sclerostomidæ of Horses and Donkeys in Egypt." *Rec. Egypt. Gov. Sch. of Med.*, I., pp. 38-40.

Railliet, A., 1895.—" Traité de Zoologie Médicale et Agricole," 2e édition, pp. 434-435.

Journal of Helminthology, Vol. IV., *No. 2, May,* 1926, *pp.* 69-74.

Ozolaimus Dujardin, 1845 (= *Macracis* Gedoelst, 1916), a little known Nematode genus from the cæcum of *Iguana tuberculata*.

By Gobind Singh Thapar, M.Sc., Ph.D., F.R.M.S.

of the Zoological Department, The University, Lucknow, India.

(*From the Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

Rudolphi (1819) first described a nematode from the intestine of *Iguana tuberculata* under the title, *Ascaris megatyphlon*. Since then considerable progress has been made in our knowledge of the parasitic Nematodes and most of the forms described by Rudolphi under Ascaris have been placed in new genera. Dujardin (1845) re-examined these forms and created a new genus, *Ozolaimus*, for *Ascaris megatyphlon* and removed certain other forms to the genus *Oxyuris*. He differentiated the two genera of *Oxyuris* and *Ozolaimus* by the presence of two simple lips in *Ozolaimus* and three simple or lobed lips in *Oxyuris*.

Schneider (1866), however, did not recognise Dujardin's genus *Ozolaimus* and merged it in the genus *Oxyuris*. Thus a certain amount of confusion prevailed about the nomenclature and both names were used for the same form. Von Linstow (1902) described a new species, *Oxyuris monhystera*, a form with two lips, and later the same author (1906) described another new species with two lips, *Oxyuris cirrata*, both being from *Iguana tuberculata*. *Oxyuris cirrata* was later transferred to the genus *Ozolaimus*, while recently Gedoelst (1916) erected a new genus, *Macracis*, for Von Linstow's *O. monhystera* and designated it as *Macracis monhystera*. It is rather unfortunate that Gedoelst gives neither the generic diagnosis nor the description of this form under the new name. It seems probable that he agrees with Von Linstow's

original description and regards that account of *O. monhystera* as representing the generic diagnosis of his new genus, *Macracis*. It would thus appear that a considerable confusion prevails. It seems necessary to clear up this confusion in nomenclature. The present paper attempts to elucidate certain points in this connection and gives an account of the general morphology of *Ozolaimus*. In my investigations, I find that the agreement of the measurements and the accounts given in different papers show the identity of all these forms under different, titles, with Dujardin's species, *Ozolaimus megatyphlon*, and they are treated as such in the following account.

OZOLAIMUS MEGATYPHLON (Rud., 1819), Dujardin, 1845.

Synonyms :—

Ascaris megatyphlon (Rudolphi, 1819).
Oxyuris megatyphlon (Rud., Schneider, 1866).
Oxyuris cirrata (Linstow, 1906).
Ozolaimus cirratus (Linstow).
Oxyuris monhystera (Linstow, 1902).
Macracis monhystera (Linstow, Gedoelst, 1916).

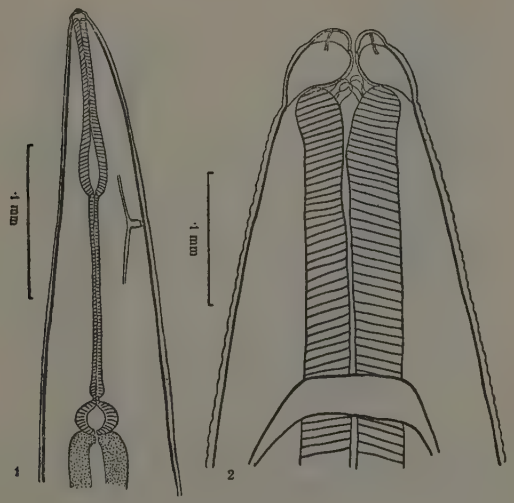
The body is fairly stout, elongated, cylindrical, and tapers towards the posterior extremity. The female is larger than the male.

The cuticle is thick and is transversely striated, and the lateral areas are well developed. The excretory pore is variable in position. It is sometimes in front, just behind the first part of the œsophagus. At other times it is behind the œsophageal bulb. As a rule, it appears to be more anterior in the female than the male, lying at about 1.45 mm. in the female and 2 mm. in the male from the anterior end. It leads into a cylindrical excretory vesicle which is connected to the four excretory canals, two running forwards and two backwards in the lateral lines.

The head is distinctly marked off from the body. The mouth is bounded by two prominent lips, each bearing a cephalic papilla. There is a very short buccal cavity. The œsophagus is very long and is divided into two parts. The first part is a short, thick, club-shaped structure, about 1·15 mm. long. This is followed by the second part which is a slender, elongated tube, joined to a sub-spherical bulb by a short narrow neck. Arising from the anterior end of the œsophagus, there are

flattened leaflike structures with knobbed extremities, that project into the buccal cavity. The intestine is rectilinear and is greatly swollen at its anterior end just behind the bulb. Posteriorly, it opens into a short narrow rectum. The nerve ring surrounds the first part of the œsophagus ·275 mm. behind the anterior end of the body.

The *female* is about 7.75 mm. long and has a stout body with a maximum diameter of ·85 mm. Posteriorly it ends in an elongated tail about ·48 mm. long. The anus is ·48 mm. in front of the tip of the tail. The vulva is protruded and is situated in the last quarter of the body, being 1·7 mm. in front of the tip of the tail. The vagina



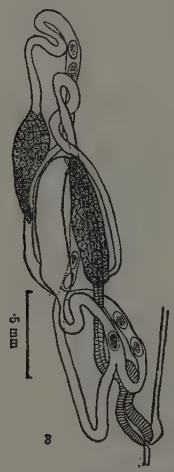
Ozolaimus megatyphlon.

Fig. 1.—Anterior end, lateral view, showing general anatomy.
Fig. 2.—Head end, greatly enlarged.

is long, slightly swollen near its opening and is directed cephalad. It joins a backwardly directed reservoir which narrows behind and then divides into two uteri. The uteri soon curve forward and may be twisted back on themselves. The ovaries are elongated club-shaped

structures. The eggs when fully developed are elongated and measure 120μ by 60μ. At the time of laying they are in a fairly advanced stage of segmentation.

The *male* is smaller than the female, being 5.7 mm. long with a maximum diameter of ·55 mm. across the level of the bulb. The body is slightly arched at the posterior end and terminates in a short tail about ·11 mm. long. On either side of the tail the cuticle is thrown out into alæ.



Ozolaimus megatyphlon.

Fig. 3.—Female genitalia. Fig. 4.—Female tail, lateral view.

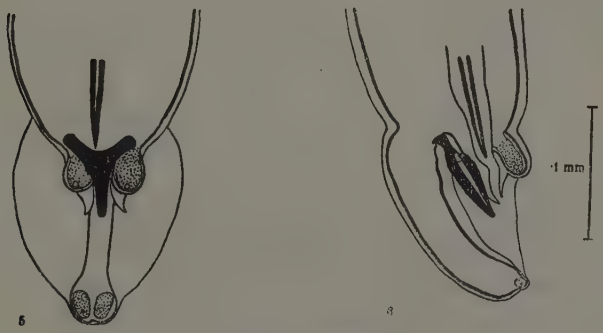
There is only one pair of genital papillæ, pre-anal in position. At the tip of the tail there is a small pair of caudal papillæ. The cloaca has two protruding lips. The anterior lip is split up into two lateral pointed lobes projecting beyond the pre-anal papillæ, while the posterior lip protrudes out as a narrow elongated and pointed structure. There is a single long acicular spicule, varying in length between 1.3 mm. and 1.4 mm. The accessory piece is about ·075 mm. long, having the form of a wide stout V.

Discussion.

We have now to consider the systematic position of the genus, *Ozolaimus*, the prominent features of which, from the above description of the only species contained in it, are :—

Mouth with two lips only. Œsophagus consists of two distinct parts joined on to a posterior bulb. Vulva protrudes and the ovejector is absent. In the male, there is one pair of genital papillæ ; a single needle-shaped spicule and an accessory piece ; caudal alæ are present.

It is evident that the genus shows affinities with the Spiruroidea on



Ozolaimus megatyphlon.
Fig. 5.—Posterior end of male, ventral view.
Fig. 6.—Posterior end of male, lateral view.

one hand and the Oxyuroidea on the other. In the possession of two lips it is allied to the Spiruroidea, but we find that certain genera of Spiruroidea are even without lips, whereas there is reduction of lips even in Oxyuroidea and sometimes we find forms with two lips only. So that on the basis of this character alone we can classify it under either of the two superfamilies. We have, therefore, to seek some other feature of a more diagnostic nature than this, in order to enable us to ascertain its exact systematic position. Ward (1917) has based his classification on the character of the Œsophagus and later on, Ward and Magath (1917) have further shown the possibilities of division of the nematodes into several groups on this character alone.

Taking this character we find that the consideration of the peculiarities of the œsophagus would closely associate *Ozolaimus* with the genus *Oxyuris*, both having a double-bulbed œsophagus. Besides, its general morphology is on the same Oxyurid plan, more particularly in resembling the Reptilian forms. We can, therefore, safely assign the genus *Ozolaimus* a position along with the Oxyurids of Reptiles under the family Oxyuridæ. The presence of two lips may be due to reduction, as is met with in certain other genera of Oxyurids.

A further remark may be added about the relationship of the genera *Alæuris* and *Ozolaimus*, as the two genera show a superficial resemblance, more particularly in the presence of the caudal alæ in the males of both. A closer examination, however, reveals that the two genera are notably distinct. The chief features in which they differ from each other are :—

1. Two lips in *Ozolaimus*, instead of three in the *Alæuris*.

2. Division of œsophagus in *Ozolaimus*, into an anterior and a posterior region and its connection with the posterior bulb by a short neck. In *Alæuris* no such division, the œsophagus being simple, cylindrical and joined to a posterior bulb by a short neck.

3. In *Ozolaimus* no ovejector which, however, is present in *Alæuris*.

4. Only one pair of genital papillæ in *Ozolaimus*, instead of three in the genus *Alæuris*.

References.

Dujardin, F., 1845.—" Histoire Naturelle des Helminthes vers Intestineaux," pp. 136, 145-147. Paris.

Gedoelst, L., 1916.—" Notes sur la Faune parasitaire du Congo Belge." *Rev. Zool. Afric.*, vol. v., Part 1, pp. 76-77.

Linstow, O. Von, 1902.—" Atractis cruciata und Oxyuris monhystera, zwei neue Nematoden aus Metopoceros cornutus " (= Iguana tuberculata). *Centralbl. für. Bakt. Parasit und Infectionskunde.*, Bd. xxxi., pp. 28-32.

Linstow, O. Von, 1906.—" Nematoden des zoologischen Museum Königsberg." *Arch. für. Naturg.*, Bd. III., Heft 2, pp. 249-258.

Rudolphi, C. A., 1819.—" Entozoorum Synopsis," pp. 285.

Schneider, A., 1866.—" Monographie der Nematoden," pp. 111-125. Berlin.

Thapar, G. S., 1925.—" Studies on the Oxyurid Parasites of Reptiles." *Journ. Helm.*, vol. III, No. 3, pp. 118-122.

Ward, H. B., 1917.—" On the Structure and Classification of North American Parasitic Worms." *Journ. Parasit.*, vol. iv., pp. 1-11.

Journal of Helminthology, Vol. IV., *No.* 2, *May*, 1926, *pp.* 75-86.

Observations on *Strongyloides fülleborni* von Linstow, 1905, With Some Remarks on the Genus Strongyloides.

By T. Goodey, D.Sc.

Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.

Introduction.

In some investigations on skin penetration by the filariform larvæ of Strongyloides (Goodey, 1925), the writer made use of larvæ cultivated from the droppings of a Guinea Baboon, *Papio papio*, which had died at the Gardens of the Zoological Society of London. Scrapings of the lining of the small intestine of this animal were made so as to secure the parasitic worms of which 25 specimens were obtained. A study of these was undertaken because von Linstow's (1905) original description of the species is so slender and his figures illustrating it are so crude that it was considered that the parasite would repay further investigation. Moreover one or two of his statements as, for example, that the cuticle of the parasite females is unstriated, required critical examination. Again in a recent paper Chandler (1925) has thrown doubt on the specific identity of *S. fülleborni*, suggesting that it is probably a hostal variety of *S. papillosus*. For these reasons the present paper has been prepared in that it gives a fuller account of *S. fülleborni* both in the parasitic and in the free-living generation than has hitherto been available. The writer considers that a detailed and well illustrated account of the morphology of *S. papillosus*, particularly of the free-living, sexual generation, is much needed at the present time.

The Parasitic Generation.

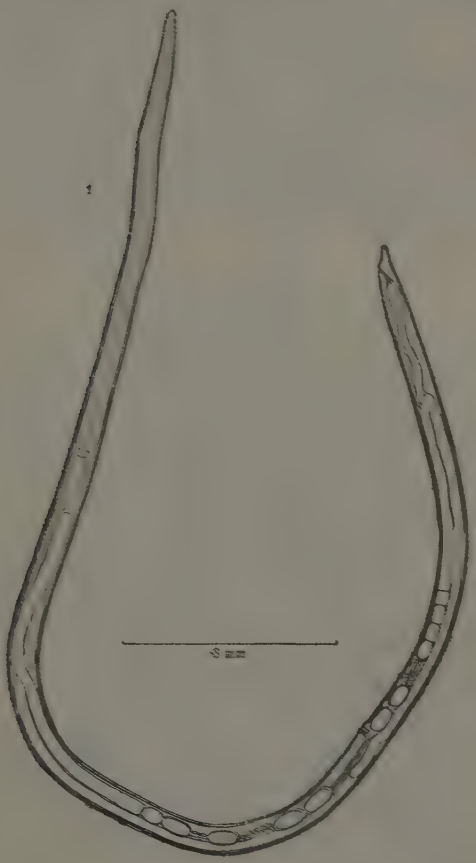
The scrapings from the small intestine were shaken up with normal saline and portions of the resulting emulsion were carefully examined under the binocular microscope for the parasite. Altogether 25 specimens were collected and fixed in hot 70 per cent. glycerine alcohol after first being well washed in saline and later these were mounted in glycerine for microscopic examination.

Von Linstow said that the cuticle is smooth and this fact has been commented on in two recent papers. Chandler (l.c. p. 431) suggests that it would be surprising if one species of the genus should be found to be free from striations when all the other known species have striated cuticle. Sandground (1925, p. 62) says that he found the cuticle to be very finely striated ; the striations often being only resolvable under an oil-immersion objective. The writer has also found that the cuticle carries very fine striations and there can therefore be no doubt that von Linstow's statement that it is smooth was an error probably due to his not examining the worms under sufficiently high magnification. Von Linstow gave 3.78mm. as the length of the parasitic worms, whereas Sandground says that his sexually mature specimens ranged from 2.02-2.85 mm. in length. The lengths of the specimens examined by the writer, all sexually mature forms, agree remarkably closely with Sandground's figures, as they form a gradual series from 2.03-2.96mm. and in view of this it seems possible that von Linstow set down 3·78mm. in error for 2.78mm.

There are six small papillæ round the mouth and the tail is bluntly rounded and finger-shaped ; a character it possesses in common with all the known species of the parasitic generation of Strongyloides except that recently described by Chandler from the cat in Bengal, *S. felis*.

The œsophagus varies in length from 0.65-0.92mm., and it is worthy of note, in passing, that the longest œsophagus does not occur in the longest worm, as is shown by the following examples which give in mms. the total lengths and lengths of œsophagus respectively in three worms :—2.03, 0.7 ; 2.47, 0.92 ; 2.87, 0.71. Roughly it may be said to vary from $\frac{1}{3}$ to $\frac{1}{4}$ of the total length. It is without a posterior swelling and contains well developed œsophageal glands.

The vulva is situated well posterior to the middle of the body which it divides approximately in the proportion of 2 : 1. The gonad is



Strongyloides fülleborni.

Fig. 1.—Entire worm of the parasitic generation showing principal anatomical features. The beginning and end of the intestine only are shown whilst the posterior loop of the ovary is figured as of the hair-pin bend type, which is not found in the majority of this species.

double and is composed of an anterior and a posterior loop. Sandground (l.c. p. 65) has called attention to the condition of the ovarian loops in the parasitic generation of worms of this genus and shows that they are either twisted or simple hair-pin bends. He also records that in all the examples of *S. fülleborni* examined by him both anterior and posterior loops are twisted. In the specimens examined by the writer the majority had both loops twisted whilst one or two had the posterior loop of the simple hair-pin bend type. In no case examined has the posterior loop been found to be so coiled as the anterior one. Close examination of the gonad under high powers has failed to reveal any region which can be considered as a receptaculum seminis in either anterior or posterior loop of the ovary.

The Free-living Generation.

Measurements, *Females*, total length, ·95-1·05mm., width at vulva, .054-.058mm., width immediately behind vulva, .037-.039.mm, anterior end to vulva, .475-.52mm., vulva to anus, .35-.38mm., anus to tip of tail, .13-.145mm., œsophagus including vestibule, .1-.112mm., *Males*, total length, .86-.9mm., greatest width, .038-.04mm., length of spicule, .037mm., length of accessory piece, .019mm.

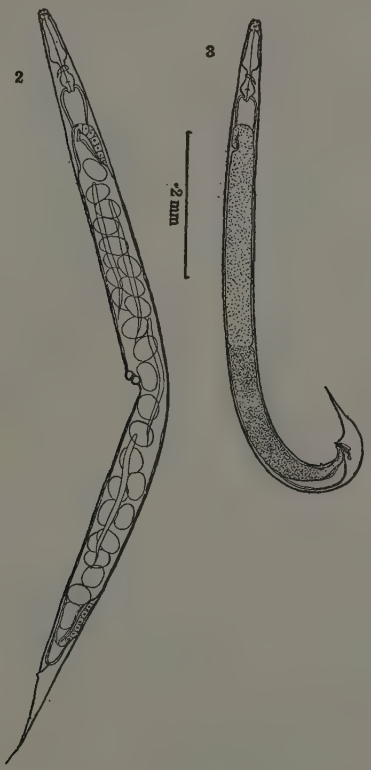
In cultures made from the fæces taken from the rectum, large numbers of males and females occurred in the first day or two. The males gradually died off, but the females attained full growth with their eggs developing into larvæ, so that old forms were seen to possess numerous free larvæ and eggs containing coiled embryos in the uterus.

The head carries 6 papillæ, four of which are fairly distinct, two subventrally and two sub-dorsally situated. The female tail gradually tapers to an extremely fine point, much finer than that of the female tail of *S. stercoralis*, whilst the male tail is always curved inwards towards the ventral surface and tapers to a fine point posteriorly to the post-anal papillæ.

The most noticeable feature of this generation is the marked constriction of the female body immediately posterior to the vulva, giving it a waist-like appearance. In many cases the narrowing is so pronounced that the genital opening is directed posteriorly, *i.e.*, almost

parallel to the longitudinal axis of the worm and not at right angles to it.

There are one or two details of structure in both sexes not adequately or accurately figured by von Linstow, namely the oesophageal region and the male spicules and accessory piece. These are dealt with in detail in the following account.



Strongyloides fülleborni.

Fig. 2.—Female of free-living generation showing in outline the salient features; note the post-vulvar constriction of the body causing the vulva to become backwardly directed.

Fig. 3.—Entire male of free-living generation under low power magnification.

The mouth leads into a vestibulum or pharyngeal portion, the walls of which are convex in outline whilst the lumen has anterior cuticular thickenings. The œsophagus occupies about 1/9 to 1/10 of the total length of the body; von Linstow gave it as 1/6. It is made up of two distinct parts connected by a narrow neck. The anterior part is almost cylindrical in shape and somewhat swollen just before it narrows down to the neck. The latter is rather short and is followed by the flask-shaped swelling in the centre of which the cuticularised valves can easily be seen. The nerve ring crosses the neck just in front of the second œsophageal swelling and not immediately posterior to the first portion as figured by von Linstow. The various parts are shown in Fig. 4, and for the purpose of comparison and differentiation the corresponding region in *S. stercoralis* is shown in Fig. 5, drawn to the same scale. This drawing was made from a worm judged to be in the same state of maturity as the female of *S. fülleborni* the anterior end of which is shown in Fig. 4, both contained larvæ free in the uterus in addition to numerous embryonated eggs. The figures show that the neck connecting anterior and posterior parts of the œsophagus is much shorter in *S. fülleborni* than in *S. stercoralis*. It has been found in all the specimens examined of both sexes, and it is clear that it furnishes a character for the differentiation of the two species in question.

The male gonad is single and extends anteriorly almost as far as the œsophagus, where it turns backwards for a short distance. Throughout most of its length it occupies practically the whole width of the body. The vesicular seminalis is set off from the anterior, testicular portion by a distinct constriction and the terminal part is narrowed down to form a well-defined duct leading to the cloacal opening. There are two pairs of caudal papillæ; a preanal pair, ventral in position and a postanal pair, dorsally situated, whilst the cloacal opening is placed on a rounded prominence. The spicules and accessory piece are not shaped as shown by von Linstow, but have more the appearance shown in Fig. 6, where only one spicule of the pair is figured.

Each is shaped like a curved knife having a broad blade with a short constricted handle swelling out into a kind of flattened knob. Extending from the forward projection at the base of the handle to the point of the spicule is a transparent expansion corresponding to the sharpened

edge of the knife. The accessory piece is shorter than the spicules, and has an irregular oval shape with a rounded distal protuberance. Owing to the fact that the spicules are situated at an angle to the dorso-ventral axis of the body, it is difficult to measure them accurately. The writer found them to be approximately .037mm. long, which agrees fairly well with the measurement given by von Linstow of .039mm.

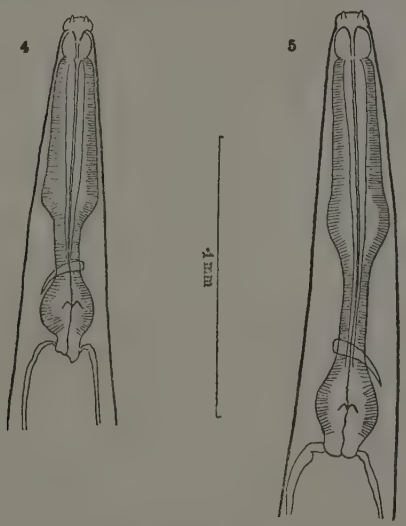


Fig. 4.—*Strongyloides fülleborni*. Anterior end of female showing disposition of the vestibule and the three regions of the œsophagus. The neck portion is comparatively short and the nerve ring lies across it posteriorly.

Fig. 5.—*Strongyloides stercoralis*. Anterior end of female for comparison with Fig. 4. Note the much longer neck portion.

For comparison, as in the case of the œsophagus, a drawing of the male tale of *S. stercoralis* is given in Fig. 7, drawn to the same scale as Fig. 6. It can be seen that both are built on the same general plan ; the caudal papillæ have the same arrangement and the spicules and accessory piece are essentially similar. In *S. stercoralis*, however, each spicule is rather longer than in *S. fülleborni* and has a somewhat longer handle portion. The accessory piece also shows a slight difference in

that the distal protuberance is more elongated and prominent than in *S. fülleborni.*

These differences are slight, but the writer has found them constant Looss(1911, p. 215) writing of Strongyloides occurring in various mammals, said. "The free-living generations, on the contrary, so far as I am acquainted with them, show slight but distinct differences from one another, which make it probable that different species exist." The observations recorded above support Looss' contention and additional evidence in the same direction is afforded by a tracing of the male tail of *S. ratti,* which Dr. J. H. Sandground kindly sent to the writer with permission to use it. It is reproduced in Fig. 8, where it can be seen that the preanal papillæ are more anteriorly placed than in either *S. fülleborni* or *S. stercoralis,* and also that the accessory piece is rather differently shaped from that in these two species.

Some Remarks on the Genus Strongyloides.

Two recent papers on the genus Strongyloides are of considerable interest, *i.e.,* those by Chandler and Sandground already mentioned several times in the foregoing pages. Broadly they may be said to give respectively a synthetic and an analytic view of the genus, for whereas Chandler suggests the arranging of all the species into two groups, viz : *stercoralis* and *papillosus,* Sandground on the other hand, although ruling out certain species on the grounds of synonymy, recognises others as valid, and creates two new ones. The latter author has also discussed very thoroughly the question of specific identity, and it is not the writer's intention to go over the whole of the ground again, but to make some comments on the present state of our knowledge of the genus in the light of these two papers and his own observations.

In the *papillosus* group Chandler provisionally places as sub-species or hostal varieties, the following species, *papillosus, ovocinctus, fülleborni, cebus, simiæ* and probably *suis,* whilst in the *stercoralis* group the following species are placed, *stercoralis, nasua, canis* and *felis.* Examining these groups in the light of Sandground's paper we see that on the grounds of synonymy they become much reduced ; *ovocinctus* and *suis* go as synonyms of *papillosus* and *simiæ* goes as a synonym of *cebus,* the group consisting of three species *papillosus, fülleborni* and *cebus.* Similarly, in the *stercoralis* group *canis* is removed as a synonym of *stercoralis* and Sand-

ground, after examining the type material of *nasua*, found it very difficult to separate it morphologically from *stercoralis*. The group therefore probably consists of two species, *stercoralis* and *felis*.

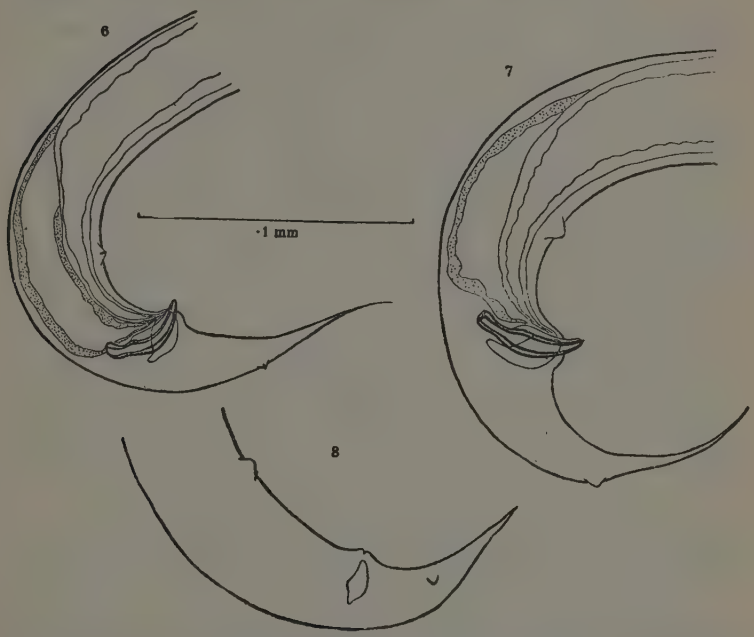


Fig. 6.—*Strongyloides fülleborni*. Posterior end of male of free-living generation showing spicule, accessory piece, pre and postanal papillæ, intestine and vas deferens.

Fig. 7.—*Strongyloides stercoralis*. Posterior end of male for comparison with Fig. 6.

Fig. 8.—*Strongyloides ratti*. Posterior end of male taken from a tracing by Dr. J. H. Sandground, showing pre and postanal papillæ and accessory piece (The original drawing was made under fairly high power magnification, but no scale of magnification was given.)

If we examine some of the points on which Chandler arranges the species into his two groups, we shall see that little value can be attached to them.

1. *Total length of the parasitic stage.*—In his *papillosus* group he gives the total length as usually between 3 and 4mm., sometimes reaching 6mm., in the *stercoralis* group the length is given as usually between 2 and 3mm. *S. fülleborni* according to Sandground and the writer's, measurements, has a total length of less than 3mm. and could equally well, therefore, be placed in the *stercoralis* as in the *papillosus* group.

2. *Shape of the tail of the parasitic stage.*—Chandler would group together those worms having a sharp constriction of the body immediately behind the anus with the tail ending in a short, bluntly-rounded finger-like tip, these forming his *papillosus* group, whilst in his *stercoralis* group he places those worms in which the body tapers evenly from some distance in front of the anus, the tail tapering evenly to a conical point, blunt at the tip. Sandground, on the other hand, found that he could not use the shape of the tail as a distinguishing character as it is subject to considerable variation. Chandler's species, *S. felis*, from the cat, is in the writer's opinion, unique in possessing an evenly tapering, pointed tail ; all the other known species have bluntly rounded finger-like tails. The tail of *S. stercoralis* is as rounded and finger-like as that of *S. papillosus*, at any rate, the outline drawings given by Sandground in his Plate VIII show that the species cannot be separated from one another on the shape of the tail. It is perhaps a little unfortunate, therefore, that Chandler should have selected *stercoralis* as the type of one of his groups on the shape of the tail.

3. *Length of tail of free-living female.*—Members of the *papillosus* group are said by Chandler to have the tail more than 1/10 the total body length, and in the *stercoralis* group less than 1/10 that length. The tails of the free-living females of *S. fülleborni*, examined by the writer, are from 1/7 to 1/8 the total length and consequently fall into the suggested group, but *S. stercoralis* in the same way can equally well be placed in the *papillosus* group, for the female tails in the writer's material are from 1/8 to 1/9 the total length.

4. *Papillæ of male tail.*—In the *papillosus* group, Chandler suggests that the postanal papillæ are (always ?) present, whereas in the *stercoralis* group they are (always ?) absent. Looss (1911 Pl. 14, Fig. 154) figures the male of *S. stercoralis* and shows postanal papillæ. Fig. 7 in the present paper shows that the males of this species examined

by the writer also possessed them. On this point then *S. stercoralis* could equally well be placed in the *papillosus* group. The presence of pre and postanal papillæ on the male tail is probably a generic character. They are certainly present and well defined in *S. stercoralis*, *S. fülleborni* and also in *S. ratti*, as the tracing by Sandground reproduced in Fig. 8 clearly shows. If they are not present in *S. felis*, and Chandler says that he could not see them, their absence is somewhat surprising, but affords another character which distinguishes this species from most of the others. Chandler would reduce the difference between *S. cebus* and *S. fülleborni* to the alleged absence of striations on the cuticle of the parasitic generation of the latter. He omits to mention, however, the waist-like post-vulvar constriction of the body in the free-living female of *S. fülleborni*, which is so characteristic of this species. Sandground found that all the females examined by him had this feature, and it was constantly present in all the specimens studied by the writer. There can be no doubt, therefore, that it is a valuable and distinctive specific character giving the worms an appearance quite different from the females of other species, in which occasionally the body is rather pinched-in just behind the vulva.

On the relative importance of the study of the two different generations of the members of the genus Strongyloides, various investigators hold different views. Chandler ends his paper by saying :—" It appears evident that the morphology of the parasitic parthenogenetic females, and their biological characteristics, are more reliable for the differentiation of the various forms of *Strongyloides* than is the morphology of the free-living adults. The latter are more variable and show less marked distinguishing characters." The opposite view as expressed in the words of Looss has already been given above on p. 82, and in the writer's opinion there is still much to be said in favour of that view. The study of the free-living generation in any case where this can be done should be undertaken in as great detail as possible with the use of oil-immersion lenses coupled with the making of careful camera lucida drawings. If this were done slight but constant differences of structure would probably be revealed as the writer has found in his study of the free-living stages in *S. fülleborni* and *S. stercoralis*.

At the present time we may with safety recognise that certain wel defined species possessing constant morphological differences exist, and that some of these species are able to parasitise, either naturally or by experimental means, more than one host as is exemplified by *S. stercoralis*, occurring naturally in man and dogs, *S. fülleborni* occurring naturally in several of the Catarrhine Primates and *S. papillosus* which occurs naturally in sheep and can be experimentally transmitted to rabbits and guineapigs.

References

Chandler, Asa C., 1925.— "The Species of *Strongyloides* (Nematoda)." *Parasitology*. 17. No. 4, pp. 426-433.

Goodey, T., 1925.—"Observations on certain Conditions Requisite for Skin Penetration by the Infective Larvæ of Strongyloides and Ankylostomes." *J. Helm.* 3. No. 2, pp. 51-62.

Von Linstow, 1905.—"*Strongyloides fülleborni* n.sp." *Centralbl. f.Bakt.* 1 Abt. Originale. Bd. 38 Heft, 5, pp. 532-534.

Looss, A., 1911.—"The Anatomy and Life History of *Agchylostoma duodenale. Dub.*" Pt. II. The Development in the Free State.

Sandground, J. H., 1925.—"Speciation and Specificity in the Nematode Genus Strongyloides." *J. Parasit.* 12. No. 2, pp. 59-80. Full list of references given in this paper.

Journal of Helminthology, Vol. IV., *No.* 3, *August*, 1926, *pp.* 87-114.

Heterodera schachtii (Schmidt) and Soil Acidity.

By B. G. Peters, B.Sc.

Ministry of Agriculture Research Scholar.

(*From the Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Introduction.

By reason of its very fertile soil, South Lincolnshire is an important potato-growing district, noted particularly for its Early varieties. However, in recent years the crop has seriously diminished and efforts have been made to discover the reason for this.

Mr. D. O. Morgan, B.Sc., of the Institute of Agricultural Parasitology, went to South Lincolnshire in 1924 and investigated the problem for some twelve months. During this time he collected several samples of soil from experimental and ordinary plots, and of most of these he estimated the content of eelworm cysts.

Reporting on his recent investigations in the locality, Morgan says: "The stunted growth of the plant, together with the wilting and yellowing of the foliage, usually associated with root trouble, were the symptoms observed, while the yield suffered greatly owing to the small size of the tubers formed. In a few instances the crop over the whole field was so poor that the yield hardly paid for the seed planted; others shewed bad patches which seemed to increase in size year after year.

"This failure of the crops had been attributed to various fungoid pests which had been found on the plants examined, and it was not until 1924 that the finding of large numbers of cysts of *Heterodera schachtii* on the roots drew attention to this eelworm as a possible cause of the trouble. Early and second early varieties of potatoes seemed to suffer most. . . ." *

* "Investigations on Eelworm in Potatoes in South Lincolnshire." p. 185.

On the instruction of Professor Leiper and by courtesy of Mr. Wallace, Principal of the Kirton Agricultural Institute, the writer joined Morgan at Kirton in October, 1925; as the possibility of some correlation between the distribution of eelworm cysts and the acidity of the soil had seemed to Morgan worth investigating. A large number of the samples had been taken by Morgan from plots upon which he was studying the alleged correlation between the incidence of failure in potatoes and eelworm infestation. The soil acidity of the various samples was afterwards determined at the Institute of Agricultural Parasitology at St. Albans.

Crowther (1922) had pointed out that while soil acidity has in the past been usually determined by " lime-requirement " methods, these latter give the " quantity factor," *i.e.*, the total amount of available acid as measured by titration. Whereas of greater importance is the " intensity factor," *i.e.*, the hydrogen ion concentration. In connection with total-acidity methods, Fisher (1921) states that the results obtained for a single soil sample by different methods vary considerably, some being as much as twenty times the value of others. Hence it seemed advisable to employ a " hydrogen ion concentration " method in the present instance.

This paper, then, contains a description of and a discussion on hydrogen ion concentration-tests of the over-mentioned soil samples, and an analysis of the results with reference to a possible correlation with eelworm cyst distribution.

Note on *Heterodera schachtii.*

The potato-root eelworm, *Heterodera schachtii* (Schmidt), is a microscopic nematode parasitic in the roots of potatoes and other plants. As such it is to be distinguished from *Tylenchus dipsaci*, an eelworm which (in potatoes) is parasitic in the tubers themselves (Goodey, 1923).

According to Shaw it has been known since 1859 (Schacht), and by 1878 was recognized as the cause of the " beet-weariness " (Rübenmüdigkeit) which was devastating the sugar-beet crops in Germany. Shaw refers to the potato as " little subject to nematode infestation in Europe " and suggests including it in his list of plants practically nonsusceptible to attack by *H. schachtii*. He quotes Bolte to the effect that these pests

" do not often appear on potatoes, and this tuber is generally included in the German crop rotations." It is therefore interesting to find quite heavy infestations on many of the potato-fields of South Lincolnshire.

Shaw gives a list of over 60 susceptible plants ; but in this connection the possibility of distinct " biologic strains " must be considered.

The following account of the etiology of the parasite is condensed from Shaw in part, and refers actually to the sugar-beet strain. The latter appears to be almost identical with the strain found on potatoes, however, and upon it much of the existing work has been done.

If an infected plant is lifted during the summer, on its roots may be seen a number of white spherical bodies of about 1 mm. in diameter. These are the protruding bodies of adult females, and are distended with eggs to the number of about 350. These females die and drop off. The enclosed eggs hatch, and the larvæ escape to seek the roots of the host plant. Here they bore into the peripheral portions of the root, where moulting occurs. The male becomes cylindrical, about 1 mm. in length, and possesses a well-marked anterior spear. It bores its way out of the root and travels in search of a female.

The female becomes spherical in shape, and its spear is less well developed. The vulva shifts from the ventral surface to a posterior position, and the latter region of the growing worm ruptures the outer layers of the root, while the anterior end remains fixed in the root-tissues. At this stage fertilization occurs, after which the eggs mature, the female dies, and the cycle is repeated.

As the weather grows cooler, not only these white females, but also the enclosed eggs and larvæ, perish.

To a slight extent during the summer and considerably in the autumn, however, certain of the females assume the " brown cyst " condition. The distended skin becomes brown and tough, the female dies and drops from the root, and its body becomes a resistant cyst. The enclosed eggs and larvæ, which remain passive but viable, are thus protected and preserved until the following season. It is probable, moreover, that some of the larvæ remain viable for five or six years, a few escaping from the cyst each season.

The cyst is very resistant to climatic conditions, and to heat especially. From experiments carried out by Fuchs (1911) it appears that a few larvæ remained alive after the cysts had endured a temperature of 55° C. for 12 hours. 24 hours at the same temperature proved fatal to all, as also did 1 minute at 63° C.

In the case of the beet-nematode, Shaw indicates two main results of infection on the host plant :—

(1) The reaction of the host involves the growth of complexes of giant cells in the vascular bundles of the roots. These obstruct the root and prevent the passage of the vital fluids : the death of many roots and the rapid growth of new ones are entailed, resulting in a hairy or " whiskered " beet. Yellowing and wilting of the leaves accompanies a bad infection.

(2) The damage from laceration by the eelworm lays the plant open to secondary infections through the entrance of fungoid and bacterial pests.

Note on " Hydrogen Ion Concentration."

The phenomena of hydrogen ion determination are most easily explained by the ionisation hypothesis. That there is some difficulty in reconciling the behaviour of concentrated solutions of strong electrolytes with this hypothesis is true ; but hydrogen ion determination in biological problems involves relatively weak concentrations of (usually) weak electrolytes, so that these difficulties may be neglected.

On the ionisation hypothesis, when an electrolyte goes into solution some of its molecules dissociate into two kinds of " ions "—atoms or groups of atoms bearing positive (" kations ") and negative (" anions ") electrical charges respectively. Thus a molecule of common salt would become, on dissociation, a sodium kation and a chlorine anion. This is conventionally symbolized :

$$NaCl = Na^{+} + Cl^{-}$$

A strong electrolyte is one of which most of the molecules so dissociate in fairly strong solution (*e.g.*, chemically " Normal "). A weak electrolyte

is one of which relatively few molecules dissociate in such strong solutions, and great dilution is required to ensure almost complete dissociation The importance of these weak electrolytes in hydrogen ion determination will be seen later, in relation to " buffer " solutions.

Electrolytes are conveniently divided into acids, bases and salts, of which acids and bases are by far the more chemically energetic. A molecule of an acid always contains at least one hydrogen atom, and that of a base at least one hydroxyl group (—OH). The dissociating acidic molecule yields a hydrogen kation, the remainder of the molecule becoming the anion. Similarly the basic molecule yields a hydroxyl anion, the remainder becoming the kation. The " activity " of acids and bases is ascribed to the hydrogen and hydroxyl ions respectively. Water, which dissociates very slightly as follows :—

$$HOH = H^+ + OH^-,$$

may therefore be regarded as a weak electrolyte of the nature of both an acid and a base. Since a given number of dissociating molecules will yield the same number of both anions and kations, the " neutrality " of pure water is explained. Similarly chemically equivalent quantities of an acid and a base neutralize each other.

This latter fact is made use of in titration, where a known quantity of base (or acid) is employed to determine an unknown quantity of acid (or base). Thus titration reveals the total " quantity " of available acid (or base) present, whereas the important factor in a chemical reaction is the " intensity " of the electrolyte : in other words, the concentration of hydrogen (or hydroxyl) ions present at a given time.

It is convenient to restrict the term " concentration " to " molar concentration " (*i.e.*, the molecular weight in grams of a substance per litre), and to symbolize the molar concentration of a substance by enclosing it in square brackets. Thus " concentration of hydrogen ions " will be symbolized : $[H^+]$, and $[H^+] = 1$ will imply a solution containing 1 gram of hydrogen ions per litre. (N.B.—The molecular weight of hydrogen is 2, but the molecule of hydrogen contains two atoms : it is not H_2 but H^+ which is being measured.) This will be the hydrogen ion concentration of an ideally strong acid in Normal (N) solution. The acid might be in N/1,000,000 solution however, where $[H^+] = \cdot 000,001$. Such considerable numerical variations are inconveniently large to

handle, especially in graphical form, so it will be simpler to use a logarithmic function of $[H^+]$. Moreover $[H^+]$ being nearly always less than unity, such a function will bear a minus sign. To simplify further, this can be adjusted by taking $\log. \frac{1}{[H^+]}$ instead of $\log. [H^+]$. This function, $\log. \frac{1}{[H^+]}$, is in general use and is symbolized by pH (or typographical variations of this):

$$pH = \log. \frac{1}{[H^+]}$$

As a matter of fact, in determining $[H^+]$ by the hydrogen electrode use is made of the following equation:

$$\frac{\text{Potential}}{\text{Numerical factor}} = \log. \frac{1}{[H^+]};$$

and it was Sørensen, the pioneer of hydrogen ion determination, who saw that this function of $[H^+]$ might advantageously be employed. He termed it the hydrogen ion "exponent."

Moreover, a logarithmic function more truly depicts the chemical relationship, for, as Clark points out: "The difference between 1×10^{-9} and 2×10^{-9} may be as important for one set of equilibria as the enormously greater difference between 1×10^{-2} and 2×10^{-2} is for another set of equilibria." * It may be emphasized here that pH is an inverse function of $[H^+]$: it is apt to be confusing at first to find that the more acid a solution is, the lower is its pH-value.

As already mentioned, pure water dissociates slightly. It has been found in this case that $[H^+] = 1 \times 10^{-7}$ (pH = 7) approximately. Obviously $[OH^-] = 1 \times 10^{-7}$ also, $(pOH = \log. \frac{1}{[OH^-]} = 7)$.

Since electrolytic dissociation follows the Mass Law, the product of the concentrations of the ions will vary as the concentration of the undissociated electrolyte. In the case of water:

$$[H^+][OH^-] = [HOH]\,K.$$

As the degree of dissociation is so slight, [HOH] will not appreciably change, and may be combined with K. Then:

$$[H^+][OH^-] = K_w = 1 \times 10^{-14} \text{ (approx.)}.$$

K_w is termed the "Dissociation Constant" of water.

* "The Determination of Hydrogen Ions." 1925. p. 36.

It follows that however concentrated the hydroxyl ions may be, as in a solution of a base, there will always be sufficient hydrogen ions to satisfy the above equation. Thus in the case of an ideally strong acid in Normal solution, $[H^+] = 1$ (pH = 0); and of an ideally strong base in Normal solution $[H^+] = 1 \times 10^{-14}$ (pH = 14; pOH = 0). Hence, it is not necessary to employ a pH scale for acids and a pOH scale for bases: one will do for both, and the pH scale is arbitrarily chosen for this purpose.

Thus the pH scale from 0 to 14 covers the range from ideal Normal acidity, through neutrality, to ideal Normal basicity (the pOH scale would simply be the reverse of this).

Electrometric and Colorimetric Methods.

If an electrode saturated with hydrogen is immersed in a solution o hydrogen ions, a difference in electrical potential will exist between the solution and the electrode; and this potential difference will depend on the concentration of hydrogen ions. On this fact have been based methods for determining pH, though the experimental procedure is very much more complicated than the above statement would suggest.

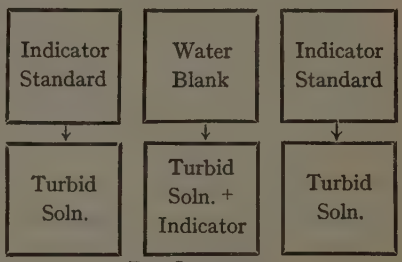
These electrometric methods admit of much greater accuracy than the alternative colorimetric methods, but they entail a very involved technique, and are open to many sources of error. Such accuracy would be wasted on a problem like the present one, and the methods are only mentioned because they supply the data which make colorimetric determinations possible.

Indicators.

Indicators for the determination of pH are substances which behave similarly to litmus. Above a certain pH they present one colour: below a certain other pH they present the alternative colour. In the region between these two pH-values they present intermediate tints from one extreme to the other and within this range they can be effectively used for pH determination. For if the pH-value corresponding to a given tint is known, it is simply a matter of matching tints. Any one indicator will only cover a limited effective range of pH (usually about 1·6), but by using a number of indicators the ranges of which overlap it is possible to cover the entire pH-scale.

Having chosen an indicator such that, when it is added to the solution under test, one of its intermediate tints is presented, the latter is matched with a standard tint the pH-value corresponding to which is known. The standard tints may take the form of a colour chart (like the excellent one included in Clark's book), but it is more effective to use solutions of known pH containing the same concentration of indicator as the solution under test. These solutions may conveniently be contained in test-tubes, the tints being viewed transversely through the tube, but care must be taken to select tubes of approximately equal diameter.

In the case of turbid solutions (like unfiltered soil solutions) colour matching may be accomplished by a device due to Walpole and called a "comparator." In this case the turbidity of the solution under test is compensated for by viewing the colour-standards through an equal depth of the turbid solution. This arrangement is illustrated diagrammatically below. It will be seen that the same constituents are present in each of the optical systems.



THE COMPARATOR (*Diagrammatic*).

Indicator standards which vary by pH : 0·2 are perhaps most useful. Thus suppose the solution under test, occupying the lower central space in the diagram, gives a tint actually corresponding with pH : 6·76. By a process of elimination it will be found that the tint is intermediate between those of standards pH : 6·6 and pH : 6·8, and the two latter will occupy the upper left and right spaces. The tint will then be seen to resemble the pH : 6·8 standard more closely than the pH : 6·6 one, and pH : 6·75 will be the estimated value. It is impracticable to estimate to less than pH : 0·05, particularly with turbid solutions.

Such a method as the above has the advantage that only thin layers of indicator are viewed through. Many indicators (particularly those of the sulphone-phthalein series recommended by Clark and Lubs) exhibit the phenomenon of "dichromatism" under certain conditions. Thus a solution of pH : 5·6 to which bromo-cresol purple is added in a

test-tube, appears green if viewed transversely, but red if viewed longitudinally, with daylight as the source of illumination. Sometimes it is the thin layer which is confusing. Thus a very acid solution (less than pH : 1) and one of pH : 7·6 with phenol red appear of similar tint if viewed transversely (pH : 7·6 is well within the effective range of phenol red, towards the alkaline end). On viewing longitudinally however, the pH : 1 solution appears yellow and the pH : 7·6 solution red, thus exposing the false similarity.

BUFFER SOLUTIONS.

The indicator standards mentioned above are prepared by adding definite quanities of indicator to " solutions of known pH." These are " buffer solutions " whose hydrogen ion concentrations are, in the first instance, determined electrometrically. They consist of varying proportions of a more acid and a more basic electrolyte, and one or both of these are usually very weak : *i.e.*, they are only slightly dissociated at moderate dilutions. Often a weak acid and one of its basic salts are the components used, and Palitzsch's " boric acid—sodium borate " buffer is a typical example of this kind. In this case, by varying the proportions of acid and salt, solutions stabilized or buffered at definite pH values are available, ranging from pH 6·7 to 9·2. Other buffer mixtures have been tested and standardized (Sørensen, Clark and Lubs, etc.), covering between them the entire pH scale.

Buffer action depends on the feeble ionisation of weak electrolytes. Thus if traces of NaOH are added to pure water, the almost complete dissociation of the former yields a large amount of OH^- ions and the pH increases (*i.e.* $[H^+]$ decreases). If, however, a weak electrolyte like boric acid (H_3BO_3) be present in the water, it will dissociate only slightly into H^+ and $H_2BO_3^-$ ions, leaving a large residue of undissociated H_3BO_3 molecules. When traces of NaOH are now added to this, the chemical equilibrium is upset and the OH^- ions satisfy most of the available H^+ ions as before. But now more of the residual H_3BO_3 molecules dissociate, in order to maintain equilibrium ; more H^+ ions are thus provided, and the whole tendency is to preserve the original hydrogen ion concentration. Solutions without this residue of undissociated molecules (as of strong electrolytes) are therefore without any stabilizing tendency, and are sensitive to slight chemical influences such as traces of alkalinity from glass vessels.

Technique.

The Samples.

The soil samples used were from two sources :—

(1) Samples from a large field where variety-trial experiments had been laid down by the Kirton Agricultural Institute. In this case the soil had been carefully air-dried and rubbed down in a mortar, and the cyst-content had been determined (by Morgan).

(2) Miscellaneous samples from various parts of South Lincolnshire. These had not been rubbed down but their cyst-contents had been determined, and they represented heavily and lightly infested patches from various fields.

In both cases the soil had been air-dried and stored in stiff paper bags for some twelve months before the pH-determinations were made. The question at once arises whether air-drying has not an effect on the pH. Unfortunately during the brief preliminary visit to South Lincolnshire apparatus was not available for testing fresh undried samples, but the writer hopes to be able to do so in the near future.

However, some work has already been done on this problem, notably by Rost and Fieger (1924). Using the electrometric method, the latter have tested a number of samples in the fresh condition, air-dried and oven-dried. In their preliminary report they publish data which show that no regular relationship can be established between degree of drying and change in pH, different soils giving different results. Thus while most soils with drying decrease in pH to varying extents, some few increase in pH. Oven-drying usually increases the effect of air-drying. Qualitative estimations by the potassium thiocyanate method usually show an increase in total acidity. Alkaline soils are subject to greater variation than acid. The average difference in pH between the fresh and air-dried conditions of nine of their representative samples is 0·44. As the South Lincolnshire soils are mostly on the acid side of neutrality, the error due to air-drying introduced into the present tests is probably less than this. And as the soils tested seem mostly of a similar geological formation (Alluvial), the error is probably fairly constant.

In view of this error, and the merely relative value of the results required in this problem, it was decided to employ a colorimetric method of hydrogen ion determination.

The Standards.

The available once-distilled water was redistilled in a glass flask and condenser with all-glass connections. This twice-distilled water was stored in bottles coated internally with paraffin wax, and alone was used for making all the solutions and for the final cleaning of all apparatus.

Clark and Lubs' buffer solutions and selected indicators were used, the former being made up from the stock solutions to pH-values varying by 0·2, and stored in 250 c.c. waxed conical flasks.

Indicator standards of the phenol red and bromo-cresol purple series were already available, but the latter proved to have become inaccurate and a new series was made : bromo-thymol blue and methyl red series were also prepared. For this purpose test-tubes of " Pyrex " glass were used. They were calibrated, and only those of closely similar diameter selected. The tubes were drawn out in the form of serum ampoules and afterwards sealed in a small flame (the less heat disturbs the glass the better). They were stored in the dark.

The Solution.

The usual method of making a soil solution is to take definite proportions of soil and water, allow the mixture to stand and to shake it up periodically. For colorimetric determinations the solution (and suspension) must not be too opaque, and filtering or centrifuging is often resorted to in order to clear it. Both Fisher (1921) and Joseph and Martin (1923) advise against filtration, for it tends to make the solution more acid than before ; and moreover there seems general agreement that filtered solutions are less buffered than heavy suspensions, and the latter are therefore usually employed in electrometric determinations (Crowther, Gimingham, etc.).

Gimingham (1923) recommends a percolation method by downward displacement in a glass cylinder of soil, 12 ins. high × 2 ins. diameter. He finds the first 50-100 cc. of percolate fairly clear ; but the later, more turbid percolate, tested colorimetrically, gave closer agreement with the electrometric result. Curiously, Joseph and Martin found that *clear* extracts gave closer agreement.

Chandler (1925) uses 12 grams of moist soil in 8 c.c. of distilled water, and pours the mixture into a collodion sac. The latter is immersed in

12 cc. of distilled water and dialysate allowed to proceed for an hour. This dialysate must, like a filtrate or clear percolate, suffer the disadvantage of being feebly buffered.

In the present instance it was resolved to make a suspension by shaking and standing for definite times and to centrifuge this. The centifugate was in most cases too opaque for immediate use and therefore dilution was resorted to at this stage. Moderate dilution has little effect on the pH of well buffered solutions.

Tests were carried out to observe the effects of various proportions of soil and water, of different times of shaking and standing, of centrifuging or filtering and of various degrees of dilution of the centifugate. It will be convenient to give the results of one of these series of tests here.

TECHNIQUE-TESTS ON SOIL SAMPLE No. 33.

Technique-tests were made before the ordinary routine tests were begun, of course ; but the following series was carried out subsequently to the latter, the sample being selected for its moderate pH-value and the clearness of its solution. The whole of this series was carried out at a temperature of 15° C.

A. Soil : Water Proportions.

1, 2, 3 and 4 grams of fine soil were added to 10 c.c. of distilled water in test-tubes which were stoppered with waxed corks. 20 vigorous shakes were given, by hand, every 5 minutes for ¼ hour. The solution was then centrifuged for 5 minutes at 20 revs. per second. The centrifugate was now diluted with distilled water in the proportion 1 : 9, indicator was added, and comparison made with the indicator standards in the Comparator (*v. supra* p. 94). The results were :—

Soil : Water Proportion.	*pH.*
1 gram in 10 c.c.	6·45
2 grams in 10 c.c.	6·45
3 „ 10 c.c.	6·5
4 „ 10 c.c.	6·5

B. Period of Standing and Shaking.

2 grams of soil in 10 c.c. of water were shaken :—

20 times every 5 minutes for ¼ hour,
20 „ 10 „ ½ „
20 „ 20 „ 1 „

Other conditions were as before. The results were :—

Period.	*pH.*
¼ hour...	6·45
½ „	6·45
1 „	6·5

C. Effect of Filtering.

2 grams of soil in 10 c.c. of water were shaken for ¼ hour and centrifuged as before. One portion was now passed through a No. 633 " Postlip " filter paper. The centrifugate was diluted with water in the proportion 2 : 8. The results were :—

	pH.
Unfiltered	6·6
Filtered	6·4

The filtered solution was very little less than equivalent to the unfiltered in opacity.

D. Period of Centrifuging.

2 grams of soil in 10 c.c. of water were shaken for ¼ hour and centrifuged for 5 and for 15 minutes respectively. A 1 : 9 dilution was employed. The results were :—

Period.	*pH.*
5 minutes	6·45
15 „	6·35

E. Degree of Dilution.

2 grams of soil in 10c.c. of water were shaken for ¼ hour and centrifuged for 5 minutes. The centrifugate was diluted with water in the proportions 1 : 9, 2 : 8, 3 : 7 and 5 : 5. The results were :—

Dilution.	*pH.*
1 : 9	6·45
2 : 8	6·6
3 : 7	6·65
5 : 5	6·8

Some additional points connected with technique may be considered here.

F. Aeration.

The pH of pure " conductivity " water has been found to be 7·07, at 18° C. (Michaelis, 1914, from determinations of the dissociation constant). The once- and twice- distilled water supplies used in the present work were tested from time to time, but while each was fairly constant throughout, each gave a result considerably removed from 7·07.

The once-distilled water varied but little from pH : 5·0. This acidity was evidently due to CO_2 in solution, for on thoroughly aerating a sample the pH rose to 6·4.

The twice-distilled water gave pH : 6·1, and after aeration pH : 6·6.

On boiling these samples with the indicator present, the pH rose again slightly as follows :—

Once-distilled water : pH=6·6
Twice-distilled water : pH=6.·9.

The indicator used here was bromo-thymol blue. These latter tests were made at boiling-point. On cooling there was a sharp rise in pH :—

Once-distilled water : pH=7·0.
Twice-distilled water : pH=7·6 (or above).

On boiling again, the pH returned to the former value ; and on cooling again, to the latter. It would perhaps be more accurate to say that the tint of the samples *corresponded with* the tint of the standards pH : 6·6 and 6·9 at boiling point, for this apparent difference in pH between the hot and normal samples is probably due to a temperature influence on the indicator as much as to an actual change in pH.

However, if boiling has no permanent effect on bromo-thymol blue, and the readings after cooling are therefore valid, a glass-alkali influence seems indicated. In any case, aeration would seem a safer way of getting rid of CO_2 than boiling *in vitro*.

Experiments were done to compare the effects of (*a*) using only aerated water in making a pH test, and (*b*) using unaerated water, reading the pH, and then aerating subsequently. In a typical experiment a soil giving a clear solution was chosen ; 2 grams in 10 c.c. were shaken for ½ hour ; the mixture was centrifuged for 5 minutes, and a 1 : 9 dilution was employed. The results were :—

	pH with and *without* subsequent aeration.	
(*a*) Aerated water used ...	6·25	6·25
(*b*) Unaerated ,, ,, ...	6·05	6·25

Thus it appears that (i.) unaerated water does affect the final pH reading, and (ii.) subsequent aeration has the same effect as antecedent aeration.

In all the technique-tests above and the routine-tests below, subsequent aeration was effected.

The method employed was as follows. The test-tube was placed in a large filtering flask which was securely stoppered. Through the stopper ran a glass tube the lower end of which dipped into the solution in the test-tube. The flask was now connected with a filter-pump, and aeration proceeded by reduced pressure. This method seemed less open to error than using foot-bellows ; for while in the latter the air has to pass through the bellows and through a length of rubber tubing, in the former it only passed through a short length of glass tubing, which could easily be kept clean.

G. Adsorption.

The second additional point in connection with technique is the possibility of adsorption of the indicator by the soil particles.

Two indicators were found sufficient for covering the pH-range of all the solutions tested : bromo-cresol purple and bromo-thymol blue. No adsorption was noticed in the case of the former, but bromo-thymol blue was definitely adsorbed by certain soils, and this effect was particularly produced by aeration. Some soils gave very turbid solutions while others, equally finely divided, gave solutions remarkably clear. The adsorption of bromo-thymol blue was almost invariably associated with the *clearer* solutions. After aerating a solution with the indicator already added a yellow deposit was observable above the level of the solution—left there by the bursting air-bubbles. Whatever the tint of the solution the deposit was always yellow. On testing the latter with ammonia it gave the deep blue colour associated with the indicator in alkaline solution.

A curious adsorption effect of methyl red, not directly connected with soil solutions, is noted below.

H. The Effect of Light on Some Indicators.

It was thought advisable to investigate the effect of light in causing most indicators to fade, in a simple qualitative test. The indicators most likely to be used in the soil determinations alone were so tested ; viz. :—

Indicator.	*pH-Range.*	*Colour Change.*
Bromo-thymol blue ...	6·0–7·6	Yellow–Blue.
Bromo-cresol purple ...	5·2–6·8	Yellow–Purple.
Methyl red	4·2–6·3	Red–Yellow.

Accordingly, a double set of solutions was prepared buffered to pH-values corresponding to the extreme colours of each indicator. One set was placed in a dark cupboard : the other was left in daylight, but out of reach of direct sunlight. The solutions were protected from external atmospheric influence by the addition of a layer of purified liquid paraffin, which the writer has found efficacious for such purposes.

At the end of 25 days no difference in tint or in depth of tint was observable between the two series, and the undarkened series was then placed so as to receive any possible direct sunlight.

At the end of 56 days (only few of which had provided direct sunlight) a difference in depth of tint was clearly apparent. Of bromo-thymol blue both the colour-forms had considerably faded (probably more than 50 per cent. decrease). The loss in intensity was scarcely observable in the case of bromo-cresol purple. Both colour-forms of methyl red had obviously faded, but not to the same extent as those of bromo-thymol blue. In no case was any change of tint seen. The tint and depth of tint of the darkened series were indistinguishable from those of freshly prepared solutions.

A curious adsorption effect was seen in the case of methyl red. The indicator in the two solutions buffered at pH : 4·2 (red colour-form) had been heavily adsorbed by the layer of paraffin, but the adsorbed form was a brilliant yellow. In the two solutions buffered at pH : 6·4 (yellow colour-form) a faint yellow tinge was only just detectable in the paraffin. In the pH : 4·2 solution which had been exposed to the light, the adsorbed yellow colour had faded equally with the red in the solution on which it floated.

J. Comparative Results from Two Indicators.

Between pH : 6·0 and 6·8, where the ranges of bromo-thymol blue and bromo-cresol purple overlap, it is possible to use either indicator.

Soil solutions whose pH-value came within this range were frequently tested with both, and substantial agreement usually obtained in the results. It was unusual to obtain a difference greater than pH : 0·1, and sometimes there was complete agreement. In the event of a difference, an intermediate value was taken.

Discussion on Technique Tests.

(A.) The proportion of soil to water over the range tested makes very little difference to the final pH. Over a larger range, covering much larger proportions of water, Joseph and Martin (1923) have obtained considerable variations. They recommend a 1 : 5 proportion.

(B.) The period of shaking and standing over the range tested also makes little difference. Most workers insist that at least 1 hour should be given, but in this instance a difference of only pH : 0·05 appears between samples standing for 1 hour and ¼ hour.

(C.) Filtering lowered the pH by 0·2, and is evidently inadvisable.

(D.) Centrifuging for a longer time reduced the pH by 0·1. Doubtless the more perfect clearing of the solution left it less well buffered.

(E.) The degree of dilution of the centrifugate apparently has a large influence, a 1 : 1 dilution making a difference of 0·35 pH-degrees as compared with a 1 : 9 dilution. It seems probable that the former gives a result closer to the true pH of the soil and ought therefore to be preferred. While it was practicable to match the tints at this dilution with this particular soil sample, however, it was quite impossible to do so with some of the more turbid solutions. Even a 1 : 4 proportion was sometimes too dense for comparison.

It is strange that the more concentrated solutions and those standing for longer times should give pH-values nearer the neutral point than the others. If the reaction of a soil is acid or alkaline, it might reasonably be expected that this effect would be increased in stronger solution, whereas here the effect is decreased. It looks as if the initial acidity of the distilled water (due to CO_2) had a permanent effect on the solution, which effect was more and more overcome in stronger solution. Yet the experiment described above (sub-section F.) seems to show that the initial acidity of the water has no permanent effect, since subsequent aeration produced the same pH-result as antecedent aeration.

(F.) Aeration has been subsequent to solution in the present tests, but it would be safer to aerate the water in the storage bottles before use in any future work. Aeration seems perferable to boiling the water, and aeration under reduced pressure to that by compressed air (since the latter usually involves the passage of the air through rubber tubing and through the compressor).

(G.) The adsorption of bromo-thymol blue, and a possible slight adsorption of bromo-cresol purple, and other indicators, need quantitative examination by means of a colorimeter. The error introduced is very slight if the colour-matching is effected as soon as the indicator is added. If the aeration of solutions proceeds after the indicator is added, it may be found necessary to add a little more indicator subsequently, to compensate for adsorption.

(H.) Indicator standards should be stored in the dark, though they will stand several hours of light without fading, provided it is not direct sunlight.

(J.) The pH-results obtained by using bromo-thymol blue and bromo-cresol purple show quite close agreement. When each of two such indicators can be relied upon it may be better to average the results, though a psychological factor enters here. For most indicators give a strong colour at one extreme (blue, purple, red) and a weak one at the other (yellow, colourless), and it is easier for the human eye to detect slight changes in the weak than in the strong colour. The ultimate procedure must depend on the special circumstances attending each determination.

In view of the different pH-values resulting from varying the many factors detailed above, it is obvious that the hydrogen ion concentration of a given soil is not determined at all. The most that can be said to be determined is the pH-value of a soil solution prepared under specified conditions. *The important thing is to keep these conditions as constant as possible for all the samples tested.* The results obtained in this way will at least have a relative value.

The procedure finally adopted for use in the routine tests was as follows. To 2 grams of dry, finely sieved soil were added 10 c.c. of twice-distilled water in a " Pyrex " test-tube. The tube was then stoppered with a waxed cork, and shaken vigorously by hand 20 times every 5 minutes for $\frac{1}{4}$ hour. The mixture was then centrifuged for 5 minutes

at 20 revolutions per second. The resulting centrifugate was diluted with twice-distilled water in the ratio 1 : 9 ; the indicator was added, and aeration resorted to until a constant tint was produced. Colour-matching was then effected in a Comparator of the Walpole type. The advantage of adding the indicator before aeration proceeds lies in the fact that the indicator shows when aeration is complete, by the tint ceasing to change. This seemed sufficient to outweigh the disadvantage of slight adsorption. All tubes used in the comparator were of practically equal diameter, and were illuminated by daylight reflected from a white surface.

Results of the Tests.

A. The Variety-trial Field.

In this field an experiment had been laid down, " To compare varieties of potatoes," by the Kirton Agricultural Institute (Exp. No. 20 in their Guide for 1924). Three sections, consisting of Scotch seed, seed from potatoes grown for one and for two years at Kirton, contained between them 47 plots of Early, Second-Early and Late varieties. The field had received an uniform dressing of compound manure consisting of 4 cwts. superphosphate 30 per cent., 2 cwts. sulphate of potash and 2½ cwts. sulphate of ammonia, per acre.

On reaching Kirton, Morgan sampled and estimated the eelworm cyst-content of the soil in this and adjoining fields. His method was as follows : " Samples of soil were taken in the field to a depth of 8 ins. by means of a soil sampler, this depth representing the normal depth of cultivation. The soil was then air-dried and rubbed down in a mortar and 20 c.c. of the fine soil remaining on the 1/60th-inch mesh was taken as the portion for estimating the number of cysts present. This latter sample was placed in a narrow-necked flask, which was then half filled with water, and after shaking for a short time more water was added until the flask was almost full, and then allowed to stand. The cysts and organic matter which floated to the surface were then poured off on to a filter paper which had been placed over a small sieve, a sharp turn of the flask whilst pouring off causing all the cysts to be washed down without the danger of any clinging to the sides. This latter difficulty in pouring off all the cysts with as small a quantity of water as possible, necessitated the use of a flask with a long narrow neck, of about 1,000 c.c.

capacity." * Six samples were taken by Morgan diagonally across each plot.

Using the technique described (*v. supra* p. 104), the hydrogen ion concentration of solutions of the samples from nine plots scattered over the field was determined by the writer. The following table shows the variety of potato grown on each plot, Morgan's figures for the cyst-content of the sample, the temperature at which the pH-determination was made, and the pH-value of the solution.

Plot No. and Name.	Sample No.	Cysts per 20c.c.	Temp. T° C.	pH.	Plot No. and Name.	Sample No.	Cysts per 20c.c.	Temp. T° C.	pH.
101 Rhoderick Dhu. (Late)	1	4	16	6·7	72 Immune Ashleaf. (Early)	1	60	12·5	6·2
	2	2	16	6·7		2	70	12·5	6·3
	3	3	16	6·7		3	61	12	6·2
	4	6	16	6·6		4	34	12	6·2
	5	10	15	6·6		5	99	10	6·2
	6	9	15	6·6		6	33	10	6·2
89 Dunvegan. (Early)	1	8	14	6·7	59 Ally. (Second Early)	1	57	14	6·55
	2	12	14	6·7		2	122	14	6·65
	3	4	16	6·7		3	82	14	6·5
	4	5	16	6·7		4	90	14	6·5
	5	6	16	6·65		5	30	14	6·5
	6	9	16	6·6		6	73	11	6·3
90 Eclipse. (Second Early)	1	40	15	6·4	58 Immune Ashleaf (Early)	1	129	12·5	6·0
	2	23	15	6·3		2	42	12·5	6·0
	3	38	16	6·3		3	134	14	6·1
	4	12	16	6·3		4	80	14	5·9
	5	18	Sample	missing		5	67	14·5	5·9
	6	40	16	6·35		6	71	14·5	6·0
82 Crusader. (Second Early)	1	29	13·5	6·45	61 Arran Comrade. (Second Early)	1	196	16·5	6·2
	2	32	13·5	6·5		2	136	16·5	6·25
	3	17	14	6·6		3	113	12·5	6·2
	4	33	14	6·6		4	124	12·5	6·2
	5	30	14	6·55		5	171	Sample	Missing.
	6	59	14	6·5		6	244	15	6·2
74 Ally. (Second Early)	1	74	12	6·7					
	2	9	12	6·7					
	3	61	13	6·6					
	4	13	13	6·5					
	5	53	12	6·55					
	6	49	12	6·5					

AVERAGE RESULTS FROM THE ABOVE :—

Plot No.	Cysts per 20 c.c.	pH.	Plot No.	Cysts per 20 c.c.	pH.
101	6	6·65	72	60	6·22
89	7	6·68	59	76	6·50
90	29	6·33	58	87	5·98
82	33	6·53	61	164	6·21
74	43	6·59			

*Op. cit. p. 186.

Analysis of Results.

From the table of averages it is clear that no very exact relationship obtains between the pH of the soil solutions and the cyst-concentration. If these two sets of values are plotted on a graph a very irregular curve is produced. The curve does indicate, however, an inverse relation which, if sufficient margin for error be granted, approximates to a straight line.

The relationship is brought out much more clearly on a sketch-map of the field where many more cyst-content values (from surrounding plots) can be considered. It will be convenient to discuss the pH-data and the cyst-content data separately first.

The question of possible errors in the pH-determinations has already been examined under the section on " Technique." Every precaution was taken to ensure uniformity in the technique, and the writer believes that what errors do appear are reasonably constant for the whole series.

The horizontal distribution of acidity in the field is far from random, as the sketch-map will show. The greatest acidity is seen to exist in the top right-hand corner (as viewed in the sketch), from which a solution of pH : 5·9 was obtained. The least acidity occurs in the diagonally opposite corner from which a solution of pH : 6·7 was obtained. Between these two extremes there is a fairly even gradation, with a small area of slightly higher pH in the centre of the field. It has been found possible to interpolate lines of equal pH-value (" isohydrions " they might be called) on the sketch-map at intervals of pH : 0·1. Such isohydrions run diagonally across the field from top-left to bottom-right, in what appears to be a remarkably even manner. Samples from many other plots would be necessary to establish such unbroken regularity, but they are unfortunately not available.

The samples from each plot were numbered from 1 to 6, and were collected diagonally across each plot from the bottom right-hand corner. During the estimations, when the location of the plots was unknown to the writer, it was noticed with curiosity that the later samples from each plot usually had a lower pH-value than the earlier. The regular pH-decrease from bottom to top of the field explains this phenomenon.

It must be emphasized once more that the pH-values attach not to the soil samples but to solutions of them prepared in a specified way.

Thus although all of the values obtained are on the acid side of the neutral point, the same cannot be predicated of the soil itself *in situ*. This invalidates any attempt to fix toleration-limits (or an optimum) of acidity for *H. schachtii* ; and in any case the field did not present a wide enough pH-range for this purpose.

Morgan's cyst-concentration data can now be considered.

The possible errors here are necessarily less constant. It must be remembered that Morgan was compelled to take this field as he found it when he first reached Kirton. In sampling, he could not be sure whether he was sampling in the rows or between the rows from which the potatoes had been lifted. One sample might be taken from the site formerly occupied by a badly infested plant, and the next from between two rows.

Another source of error which was beyond Morgan's control lies in the fact that several plots of Early, Second-Early and Late potatoes were lifted late in the season, while others were lifted at normal times (these are indicated on the sketch-map). Several generations of eelworm are produced in one season, and the prolonged presence of the host-plant in some of the plots would tend to produce an abnormally high cyst-concentration.

Morgan has tested his technique, and there seems remarkably little room for error in this direction.

He saw that his cyst-content figures were useless for correlation with varieties of potatoes, and had no intention of publishing them : the writer is therefore the more greatly indebted to him for permission to use and publish them here.

In spite of these sources of error Morgan was able to demonstrate some regularity in the horizontal distribution of the cysts. In his report he says :—" The figures obtained from the plots under variety trials shewed a steady decline from one side of the field to the other and merging into a corner which contained hardly any cysts. This feature, while it deprived one of the possibility of making comparisons between varieties, shewed that there was some degree of accuracy in the method of sampling." * The average cyst-content values for every plot on the field are shown on the sketch-map (at the top of each plot).

* Op. cit. p. 187.

22	26	50	28	27	28	32	65	83	59	59	43	101	144	87
			6·33						**6·22**					**5·98**
L					L	L	L			L	L	L	L	
100	*96*	*93*	*90*	*87*	*84*	*81*	*78*	*75*	*72*	*70*	*68*	*64*	*60*	*58*
6	13	31	30	24	18	33	34	45	51	76	103	130	164	64
6·65						**6·53**							**6·21**	
L	L	L	L		L	L	L	L			L			
101	*97, 98*	*94*	*91*	*88*	*85*	*82*	*79*	*76*	*73*	*62*	*71A*	*65*	*61*	*69*
4	5	9	13	7	13	14	26	44	43	49	42	56	74	76
				6·68					**6·59**					**6·50**
L	L	L	L		L	L				L	L			
102	*99, 103*	*95*	*92*	*89*	*86*	*83*	*80*	*77*	*74*	*71*	*67*	*66*	*63*	*59*

DIAGRAM OF VARIETY-TRIAL FIELD.

Upper figures give No. of cysts in 20 c.c. of soil. Lower figures in Italics are the official plot-numbers. pH-Determinations were made on heavily-lined plots : the average pH-Values are central, in bold type. Potatoes in plots marked L were lifted late in the season.

That there is a definite correlation between the cyst-concentration and the hydrogen ion concentration of the soils seems beyond doubt. The bottom left-hand corner of the field revealed the highest pH in its soil solutions, and here the cyst-concentrations were lowest. The top right-hand corner revealed the lowest pH-value, and the highest cyst-concentrations were found in the adjacent plots (60 & 61).

The gradations between the two extremes of cyst-concentration are not very regular when the averages for each plot are considered, but by taking the plots in the sketch in horizontal series of three the individual variations are reduced. These values may be presented in a diagrammatic form corresponding to the sketch-map :—

28*	28	60	58	111
16	24	37	77	119
6	11	27	45	68

Apart from the 58 value in the top line, the diagonal gradation is well marked, and may be compared with the pH-values on the sketch-map.

In view of the fact that *H. schachtii* (and other related nematodes) can adapt itself to different hosts, Steiner (1925) has pointed out that it is necessary to know the past history of a given infested field (in the matter of crops grown, etc.) before eelworm data can have much value. The field under consideration has for many years been planted with late varieties of potatoes, included in a rotation (as is the custom in South Lincolnshire with *late* varieties). Although *H. schachtii* was not reported on potatoes from this district until 1924, there is no reason to suppose that it had not been present on this host for several years. If the latter may be assumed, the eelworm would have had time to spread over the field and to select that portion the physical conditions of which best favoured its growth.

The field was level and presented no obvious variations in the nature of the soil. It therefore seems justifiable to assume that the correlation discovered represents an actual selection on the part of the eelworm of the more acid environment. This is consonant with former observations on soil nematodes. Chandler (1925), *e.g.*, notes that soil nematodes (not specified) are greatly increased in soil of high acidity (pH : 5·2–6·0).

* Neglecting a single extreme sample.

It will be noted that the error introduced by lifting some of the plots late in the season is not made obvious in the cyst-content values. Those plots with the lowest cyst-concentrations in the whole field were lifted late.

Among the miscellaneous samples was one collected from quite near the experimental field (just to the left on the sketch-map) where no eel-worm was found. The solution of this soil gave pH : 6·8 the highest pH-value for the field. It is tempting to suggest that a soil the solution of which gives pH : 6·8 is intolerable to *H. schachtii*. This is not corroborated, however, by another miscellaneous sample, the solution of which gave the same pH-value but in which a few cysts were present. It is probable nevertheless that the toleration-limit of acidity on the alkaline side is closely approached in these cases. Possibly experiments with soils artificially buffered so as to give solutions around pH : 7·0 may establish a toleration-limit.

B. Miscellaneous South Lincolnshire Soils.

The physical and chemical nature of the South Lincolnshire soils is important from the point of view of acidity and of the technique of pH-determination. A typical analysis, performed on an arable Boston soil, is quoted here from Hall. ("The Soil," 1920. p. 342.)

Mechanical Analysis.		Chemical Analysis.	
Fine gravel (above 1 mm.) :	0·0	Moisture :	2·06
Coarse Sand (1–0·2 mm.) :	0·11	Loss on ignition : ...	6·35
Fine sand (0·2–0·04 mm.) :	53·58	Nitrogen :	0·184
Silt (0·04–0·01 mm.) : ...	12·32	Potash :	0·629
Fine Silt (0·01–0·002 mm.):	10·32	Potash (sol. in 1 per cent. citric acid) :	0·017
Clay (below 0·002 mm.) :	14·66	Phosphoric acid : ...	0·189
Moisture :	2·06	Phosphoric acid (sol. in 1 per cent. citric acid) :	0·034
Loss on ignition : ...	6·35	Lime (CaO) :	0·62
Calcium carbonate : ...	0·08	Magnesia (MgO) : ...	—
		Carbonates as $CaCO_3$: ...	0·08
		Oxide of Iron (Fe_2O_3) :...	3·60
		Oxide of Manganese (Mn_3O_4) :	0·11

It will be seen that fine sand is the predominating ingredient, while the content of clay is not abnormal. The chemical analysis presents no outstanding features, except the somewhat low value for " Carbonates as $CaCO_3$." The soil is typically a light sandy loam of Alluvial origin and high fertility.

To the north-east of Boston is to be found a narrow strip of " toft " soil. This is a soil light in texture, rather dark in colour, overlying silt. It appears to have originated from marine high-tide deposits in geologically recent times. Eelworm was common in the district. The soil has a low clay-content, and its solutions were therefore remarkably clear but adsorbed bromo-thymol blue heavily. Some of these solutions gave the low pH-values which might be expected (*e.g.*, pH : 6·0) ; others gave values as high as pH : 6·8, which fact is possibly to be explained by the liming of the fields which is occasionally resorted to.

After consultation with Morgan, the writer has decided to postpone the publication of these results in detail until further work has been done.

It may be pointed out here, however, that the results are conflicting. In many cases there was marked correlation between the pH-values of the soil solutions and the eelworm cyst-content, the cysts being more plentiful in the more acid parts of the fields. In some cases no correlation was clear : widely different cyst-concentrations were associated with identical pH-values, or *vice versa*. In a few cases a reverse correlation appeared, more cysts being found in more alkaline parts of the fields.

In connection with this last phenomenon it must be pointed out that these miscellaneous fields were not under experimental control, and it was therefore difficult to obtain scientific data of their past history. At the first incidence of eelworm on a field, it usually appears in a small patch which rapidly extends. This patch might happen to be more alkaline than other parts of the field, in which case a misleading correlation would appear. Once the eelworm had become established over the field it would presumably select the more acid environment, as it appears to have done on the Institute experimental field.

One field was of considerable interest in that it revealed no sign of eelworm whatever, in spite of the fact that potatoes had been grown on it for many years without any fallowing or rotation of crops (a not

uncommon practice in South Lincolnshire with *Early* varieties). A solution of this soil gave the low vale of pH : 6·1, which elsewhere was associated with high cyst-concentration. Moreover the field was not far removed from quite heavily infested land. This curious anomaly remains unexplained at present.

Summary and Conclusions.

1. The possibility of correlating the acidity of the soil with the distribution of cysts of *Heterodera schachtii* is investigated by a colorimetric method of hydrogen ion determination. The variety of this eelworm which infests potatoes is alone considered, the material being derived from South Lincolnshire—in particular from Kirton and the district around Boston. The samples, general notes and cyst-concentration data are due to Morgan, to whom the writer is greatly indebted.

2. A brief account of the life-history of *H. schachtii* is given, and also :

3. A note on the theoretical aspect of hydrogen ions and of the colorimetric methods for determining their concentrations.

4. The technique involved is fully discussed, and after various tests is standardized as follows : 2 grams of dry, finely-sieved soil are shaken up with 10 c.c. of twice-distilled water for ½ hour. The mixture is centrifuged for 5 minutes and diluted with water in the ratio 1 : 9. A suitable indicator is added, aeration of the solution follows, and the pH-value is read by comparison with indicator-standards in a Walpole Comparator.

It is pointed out that at most the results give the pH-value of the solutions. The pH-value of the soil itself, *in situ*, is not determined, so that the results are only of relational value.

5. Results of tests carried out on samples from an experimental field at Kirton show an indubitable correlation between pH and cyst-concentration. The cysts are very numerous in samples giving solutions of pH : 6·0 ; and few in samples giving solutions of pH : 6·7.

6. Miscellaneous samples from South Lincolnshire in part confirm this correlation, but in a few cases a reverse correlation obtains.

7. The question of the actual damage done to the host-plant, directly or indirectly, by this potato eelworm is still *sub judice*. But if it should

be found that control methods are necessary, judging from the samples tested, the writer considers that a soil, such that its solution (prepared as specified) gives a pH-value of 7·0 or over, would be unfavourable to the parasite.

The adequate liming of sour soils has been advised by many agriculturalists (*e.g.*, Thomas, 1925). Were this advice followed, it is probable that the prevalence of *H. schachtii* would be greatly reduced—at least so far as concerns the strain found on potatoes.

References.

Chandler, A. C., 1925.—" Soil Acidity and the Survival of Hookworm Larvæ." *Ind. Med. Gazette*, vol. lx., p. 10.

Clark, W. M., 1925.—" The Determination of Hydrogen Ions." Williams & Wilkins Co. (Baltimore), 2nd Edit.

Crowther, E. M., 1922.—" Soil Acidity in its Physico-chemical Aspects." *Trans. Farad. Soc.*, vol. xvii., No. 2., p. 317.

Fisher, E. A., 1921.—" Studies on Soil Reaction." I. & II. *Jour. Ag. Science*, vol. xi., No. 1., p. 19.

Gimingham, C. T., 1924.—" The Colorimetric Hydrogen Ion Determination of Soils." *Jour. Ag. Science*, vol. xiii., No. 1., p. 69.

Goodey, T., 1923.—" Eelworm Disease of Potatoes caused by *Tylenchus dipsaci*." *Jour. Helm.*, vol. i., No. 5., p. 197.

Guide to Experiments and Demonstrations on the Kirton Agricultural Institute Farm, 1924.

Hall, Sir A. D., 1920.—" The Soil." John Murray (London), 3rd Edit.

Joseph, A. T. & Martin, F. J., 1923.—" The Hydrogen Ion Concentration of Heavy Alkaline Soils." *Jour. Ag. Science*, vol. xiii., No. 3., p. 321.

Morgan, D. O., 1925.—" Investigations on Eelworm in Potatoes in South Lincolnshire." *Jour. Helm.*, vol. iii., No. 5., p. 185.

Rost, C. O. and Fieger, E. A., 1924.—" The Effect of Drying upon the Acidity of Soil Samples." *Science*, No. 60., p. 297.

Shaw, H. B.—" The Sugar Beet Nematode and its Control." Undated Reprint from " *Sugar* " (Chicago).

Steiner, G., 1925.—" The Problem of Host Selection and Host Specialization of certain Plant-infesting Nemas and Its Application in the Study of Nemic Pests." *Phytopathology*, vol. xv., No. 9, p. 499.

Thomas, B., 1925.—" Lime and Liming." Reprint from *The Fertilizer, Feeding-Stuffs and Farm Supplies Journal*," Feb. 11th.

Journal of Helminthology, Vol. IV., *No. 3, August,* 1926, *pp.* 115-122.

The Effect of the Period of Service upon Parasitic Infection amongst the Cairo City Police Force.

By M. Khalil, M.D.Brux., Ph.D.Lond.

(*Professor of Parasitology, Faculty of Medicine, Cairo, Egypt.*)

The Cairo City Police is a force consisting of more than 5,300 men. They are recruited principally from the Egyptian Army Reserve or by direct conscription. They are mostly derived from the poorest classes of the agricultural population and the artisans of the towns. Any individual can be exempted from enlistment in the Army on paying £E.20, and it is only those who cannot afford to pay that sum that are enlisted if medically fit.

The Police Force may be taken as representative of the bulk of the inhabitants of Egypt, and being selected, to represent the healthiest amongst them.

The mode of life of a Cairo Policeman entails two important changes from his previous mode of life in the village. These two changes are :—

1. He is well dressed and specially well shod in the Police Service.
2. The nature of his work in the Police does not expose him to re-infection with parasites.

It is to study the effect of these two factors on parasitic infection that the present investigation was undertaken.

During the early part of 1926, it was arranged with the Cairo Governorate to examine the whole Police Force for evidence of parasitic infection.

Technique of the Examination.

The maximum number of men examined in a single day was 100. Three medical officers, two laboratory assistants and four attendants were detailed for this work. A specimen of urine and another of stool were secured from each individual. The urine was collected in a conical urine glass and allowed to settle before the deposit was pipetted and examined under a low power of the microscope.

The sample of stool was examined by two different methods :—

1. A smear is prepared selecting, if present, any part that contained mucus or blood and then examined microscopically.
2. 1 c.c. of the stool is emulsified in concentrated salt solution, passed through a wire sieve of 100 meshes to the linear inch, into an Erlenmeyer flask of 60 c.c. capacity. This is then allowed to stand for at least 20 minutes before the surface film is lifted by means of a platinum loop and examined.

In Egypt, it is indispensable to use the two methods at the same time. The floatation method is efficient in detecting very light infections with Ancylostoma while it is inefficient in detecting the ova of Bilharzia and other parasites.

The efficiency of the floatation method as done in Egypt can be gleaned from the following analysis of 75 cases in whom the ova were counted by Stoll's and by Lane's techniques. All the cases were positive by the floatation method. Lane's method failed in detecting any ova in 5 cases, and Stoll's method failed in detecting ova in 13 cases. Negative results were in every case checked by another examination.

The floatation and smear examinations were generally done by two different medical officers. It is interesting to contrast the results of the smear and floatation methods as regards the efficacy of detecting Ancylostoma ova. These results are tabulated as follows :—

Total number of cases examined	5322
,, ,, positive for Ancylostoma...	878
Detected by Floatation method	842 (95·9%)
,, Smear method...	138 (15·7%)
,, Floatation but not by Smear ...	740 (82·0%)
,, Smear but not by Floatation ...	36 (4·1%)

The deficiency of the floatation method in detecting ova in 36 cases may be ascribed to the uncertainty as to the concentration of the salt solution. This was due to the fact that no special hydrometer recording high specific gravity was available. The specific gravity of the salt solution should be ascertained before use and it must be above 1100.

Result of the Microscopical Examination.

The total number of men examined was 5,366. All of them supplied specimens of urine and stools with the exception of 44 men who supplied specimens of urine only.

Ova of ten different parasites were met with. The result of the examination was as follows :—

Urine :	Total number of specimens examined ...	5,366
	Sch. hæmatobium infection	1,156 (21·5%)
	Sch. mansoni infection	5 (0·1%)
Stools :	Total number of specimens examined ...	5,322
	Sch. hæmatobium infection	5 (0·1%)
	Sch. mansoni infection	79 (1.5%)
	Tænia saginata infection	3 (0·06%)
	Hymenolepis nana infection	26 (0·5%)
	Ancylostoma duodenale infection... ...	878 (16·5%)
	Ascaris lumbricoides infection	264 (5·0%)
	Oxyuris vermicularis infection	67 (1·3%)
	Trichostrongylus sp. infection	121 (2·3%)
	Strongyloides stercoralis infection ...	17 (0·3%)
	Trichocephalus trichiurus infection ...	7 (0·1%)

62·8% of those examined were found negative to helminth infection.

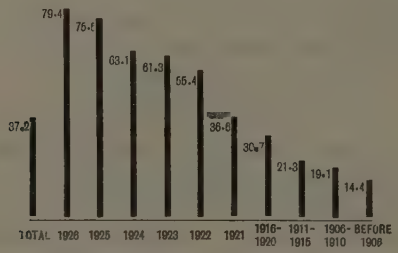
The bearing of agriculture on the incidence of parasitic infection is shown by the following result :—

	Number and percentage of infection.
Individuals previously engaged in agriculture	3,939 (41·2%)
,, indirectly engaged in agriculture (artisans of villages) ...	1,054 (28·4%)
,, artisans of big towns	329 (18·5%)

When the individuals were grouped according to the length of service in the Army and Police, the incidence of infection with helminths was found to gradually diminish, as can be seen in the following table :—

Date of joining the Service.	Number whose Urine and Stools were examined.	Found infected with Parasites.
1926 (less than 3 months)	251	199 (79·4%)
1925	540	405 (75·5%)
1924	278	181 (63·1%)
1923	178	109 (61·3%)
1922	352	194 (55·4%)
1921	338	187 (36·6%)
1916–1920	1,277	392 (30·7%)
1911–1915	811	172 (21·3%)
1906–1910	574	129 (19·1%)
Before 1906 (*i.e.*, more than 20 years.)	714	103 (14·4%)

This result is illustrated graphically in the following diagram.



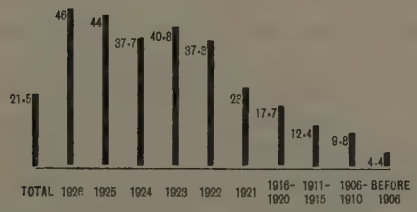
Graph 1. *Effect of Period of Service upon Parasitic Infection amongst the Cairo Police.*

It appears, therefore, that individuals infected with parasitic diseases on joining the service gradually get rid of their infection as time goes on.

Schistosoma Infection.

Sch. hæmatobium and *Sch. mansoni* are both endemic in Egypt. *Sch. bovis* is present in cattle, but so far no cases in man are recorded in Egypt.

Sch. hæmatobium infection is by far the most widespread. About 46 per cent. of the recruits were found infected. They come from all parts of Egypt. This figure however under-estimates the amount of infection in the country, as formerly, evident hæmaturia exempted the individual from conscription. Severe infection entailing pronounced weakness of body also exempts from enlistment.



Graph 2. *S. Hæmatobium Infection amongst the Cairo Police.*

Up to the present no systematic examination of the Police Force for Bilharziasis and Ankylostomiasis was carried out. Individuals complaining of the symptoms of either disease were treated. Only 178 men stated that they had received treatment for Bilharziasis by intravenous injection of tartar emetic previously, one to five years ago ; 82 per cent. of these were found to be cured and no Bilharzia ova were detected in their excreta. The other 18 per cent. were found to be still passing Schistosoma ova. No mention was made whether the ova were living or dead. The result is extremely satisfactory, in view of the long interval between the treatment previously carried out and the present re-examination.

The incidence of Schistosoma infection amongst the policemen was found to diminish gradually as the period of service increased. While 46 per cent. of the new recruits were found infected, only 4·4 per cent. of those of 20 or more years' service were infected. The accompanying graph 2 illustrates the influence of the length of period of service upon the incidence of infection. It will be noticed from graph 2 that the year 1923 is an exceptional year in that the incidence of infection is higher than that of the following year. The same anomaly is also noticed as regards Ankylostomiasis. Enquiry was made as to any special procedure followed in recruiting during 1923, but no plausible cause was found.

Schistosoma mansoni Infection.

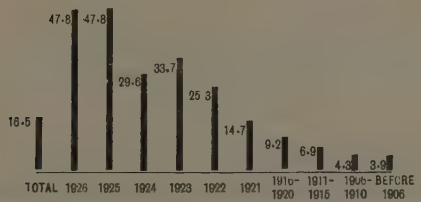
Eighty-one individuals were found to be infected with *Sch. mansoni* as compared with 1,158 cases infected with *Sch. hæmatobium*. Although 2,435 policemen came from Upper Egypt and 2,845 from Lower Egypt, yet all those infected with *Sch. mansoni* came originally from Lower Egypt. This result is in accordance with the results obtained in previous surveys carried out in Lower and Upper Egypt, and also as a result of examining about 3,000 prisoners in Tura Convict Prison. Planorbis snails were only found in Lower Egypt,whilst none were met with in the localities surveyed in Upper Egypt. The localisation of *Sch. mansoni* infection in Lower Egypt is very important. The disease caused by this parasite is much more serious than *Sch. hæmatobium* infection of the urinary tract. The Planorbis snail selects for its habitat sluggish streams which are very shallow, and in which the water is more or less stagnant. These conditions are rarely to be found in Upper Egypt. The subject demands further study.

Ancylostoma Infection.

The incidence of Ancylostoma infection amongst the Police Force decreases with the increase in the period of service. As in the case of Bilharzia infection, the year 1923 appears exceptional, the incidence of infection being a little higher than that in 1924. The attached graph 3 illustrates the incidence of infection according to the date of joining the service.

All policemen were given 5 c.c. of carbon tetrachloride. It was followed in three hours by 30 grams of magnesium sulphate dissolved in water. Of the total number of 5,366 individuals 25 complained of symptoms due to carbon tetrachloride. All these symptoms were transient and did not need active treatment. These symptoms were :—

Fainting	12	cases.
Diarrhœa	7	,,
Dizziness	3	,,
Headache and Tenesmus	1	,,
Constipation	1	,,
Colic	1	,,



Graph 3. *Ancylostoma Infection amongst the Cairo Police.*

Conclusions.

The incidence of infection with helminths in a body of selected strong Egyptian peasants was about 80 per cent. Infection with *Ancylostoma duodenale* amounts to 48 per cent. and *Sch. hæmatobium* 46 per cent.

Sch. mansoni infection is localised in Lower Egypt.

The change in life entailed by service in the Police causes a gradual elimination of parasitic infection. This is due principally to elimination of re-infection.

Bilharziasis and Ankylostomiasis can rightly be termed "agricultural diseases" in Egypt.

ACKNOWLEDGMENT.

The microscopical examinations recorded in this paper were mainly carried out by Drs. M. Abaza, A. Rabie and M. Nazmi, of the Parasitology Section of the Public Health Laboratories, to whom I wish to record my thanks for the care taken in the performance of this work.

Journal of Helminthology, Vol. IV., *No.* 3, *August*, 1926, *pp.* 123-126.

Observations on the Incidence of *Metastrongylus brevivaginatus* and *Metastrongylus elongatus* in Pigs in Central Wales.

By E. Aneurin Lewis, M.Sc.

(Demonstrator in Zoology, University College of Wales, Aberystwyth, and Field Officer, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.)

During the winter of 1921-22 I visited the slaughterhouse at Aberystwyth at least once each week for the purpose of studying the helminthic parasites of sheep and pigs in the neighbourhood. An average of 50 sheep and 8 pigs were examined weekly from November 26th, 1921, to April 28th, 1922. *Metastrongylus elongatus* and *M. brevivaginatus* were present in the bronchioles of 50 per cent. of the pigs examined, generally both species being present in the same animal, and I took the opportunity of working out the anatomy of these two forms in detail, and indicating the differences between them; but before being able to publish my results Gedoelst covered the ground in his "Le Genre Metastrongylus Molin, 1861," published in 1923. I am able, in the main, to support the account given by Gedoelst, but think the following additional notes may be of value:—

1. Gedoelst states that in *M. brevivaginatus* the spicules are striated from the distal extremity to within one-quarter of the base. This I confirm, and find the same condition obtains in *M. elongatus*, to the striation of which Gedoelst does not refer. Gedoelst refers to the spicules of the males of both species as being equal ("les spicules égaux"). This is not invariably true. They are equal or sub-equal.

2. The ease with which specimens of the two species may be distinguished from each other by the naked eye might with advantage have been more emphasised by Gedoelst. In *brevivaginatus* the male is very obviously greater. In the females the presence of the vaginal vesicle and the straight posterior extremity of *brevivaginatus* offer a clear contrast to *elongatus* in which the vaginal vesicle is absent and the posterior extremity strongly recurved. Gedoelst calls attention to the greater length of the female in *elongatus*; this I find true as of the average condition, but as shown in detail below there is considerable overlapping, which is not indicated by Gedoelst's figures.

3. Some statistical observations were made upon the width of the males and the length of the females in each of the two species, with the following results :—

Breadth of Males. One hundred specimens of each of the two species were measured. In the data obtained there is no overlapping. The average breadth of *elongatus* is ·14 mm. and its range from ·1 to ·16mm. and in *brevivaginatus* the average is ·24 mm. with a range from ·16 to ·31 mm.

Length of Females. One hundred specimens of each of the two species were measured. The average length is 30·02 mm. for *brevivaginatus* and 37·33 for *elongatus*. The former figure is in agreement with Gedoelst's observations, but the latter is a much lower average. The range of variation in *brevivaginatus* is from 19 to 37 mm., which is very similar to Gedoelst's range, but in *elongatus* it is from 12 to 58 mm., a very much wider range than that given by Gedoelst. Thus where Gedoelst finds no overlapping I find a good deal. I was careful to select only embryo-containing specimens.

4. *Occurrence and Symptoms.* Some notes were made on the occurrence and symptoms. An infested lung shows on its surface pale pink patches which are harder than the surrounding tissue. In cases of light infection the patches are confined to the margins at the end farthest away from the trachea; in heavier attacks the patches spread along the margins. In still heavier attacks they may spread more generally over the surface of the lung. They are found on otherwise healthy lungs and also on those of pigs which have suffered from colds and pneumonia.

On dissecting out the patches referred to it is found that the small bronchioles are filled with tangled masses of cream-coloured worms, bathed

in a viscid, purulent substance of a bluish-green or grey colour. The worms are so thin and fragile that it is difficult to extricate individuals. Within my experience both species are always present, but *elongatus* in the greater numbers. Free ova are sometimes found in the viscid pus, but this may have been due to the rupture of mature worms left in the lung, which was sometimes allowed to cool during washing.

The tracheæ and the larger bronchial tubes have been examined at various times, but on two occasions only have worms been found there ; these were mature.

In order to find the percentage of pigs infected the following observations were made :—

From December 13th–29th, 1921, 22 pigs were examined ; 12 were infected. From January 5th–28th, 1922, 49 pigs were examined, and 21 were infected. From February 3rd–28th, 31 pigs were examined ; 13 were infected. From March 10th–30th, 22 pigs were examined ; 11 were infected ; and from April 4th–20th, 13 pigs were examined, and 3 were infected.

It will be noted that out of 137 pigs examined, close upon 50 per cent. were infected. The number of pigs attacked remains much the same throughout the period of the investigation ; the March increase characteristic of some worms is not indicated. Throughout the period very small young Metastrongylus occur in the same bronchioles and at the same time as larger, more mature ones.

The worms were in each case counted, those from each host being kept separately.

The number of parasites per host varies within wide limits, namely, from 5 to 407 ; but the figures do not suggest a seasonal variation in frequency, and, as was pointed out above, mature and immature forms were found together at all periods of the investigation. Females are more numerous than males, and males of *elongatus* more numerous than males of *brevivaginatus*.

So far as has been observed, a post-mortem examination must take place before the presence of the Metastrongyles can be definitely determined. It has been noted, however, that the infected pigs tend to lose weight.

The pigs killed at the Aberystwyth slaughterhouse usually range in age from 4 months to 14 or 15 months. The age and weight were ascertained in the following cases for correlation with the number of worms present :—

Two pigs of 4 months, and weighing 94 lbs. and 92 lbs., contained 102 and 397 worms respectively ; four pigs of 4½ months, and weighing 100, 102, 89, 78 lbs., contained 88, 178, 214 and 417 worms respectively ; one pig of five months, and weighing 122 lbs., contained 5 worms ; one pig of 5½ months, and 103 lbs. in weight, contained 217 worms ; one of 6½ months, and weighing 191 lbs., contained 93 worms. A 7-months old pig, which weighed 160 lbs., contained 231 worms ; two pigs of 12 months, of 250 and 200 lbs. in weight, contained respectively 83 and 178 worms.

Two pigs, one of 14 months and the other of 14½ months, and each weighing 270 lbs., contained no worms at all.

The data are few, but they support one's impression that the older pigs are less heavily infected than the younger ones, and that, furthermore, heavy infections tend to be related to reduction in weight. A heavily infected pig has a very unthrifty appearance ; it looks weak and thin and is poorly developed. The Aberystwyth butchers state that if the kidneys are well hidden in fat it is an indication of good constitution and health. This condition has not been observed in animals heavily attacked by the Metastrongyles. When the sides of a heavily infected pig are cut they are not found to be thick as they would be in a pig of good quality ; they are deficient in fat. These remarks were clearly illustrated in, for example, the four month pigling with 397 worms mentioned above, and in the four and a half month pigling with 417 worms.

It should be noted that the pigs killed at the slaughterhouse are of different breeds ; some are of pure pedigree while others are crosses, but it is not thought that this is a material factor within the limits of the present investigation.

REFERENCE.

GEDOELST, L., 1923.—" Le Genre Metastrongylus Molin, 1861." *Bulletin de la Société de Pathologie Exotique*, XVI., pp. 624-630. Paris.

Journal of Helminthology, Vol. IV., No. 3, August, 1926, pp. 127-142.

On a Collection of Helminths from a South African Farm.

By R. J. Ortlepp, M.A., Ph.D., F.Z.S.

(Senior Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.)

During the winter of 1925-1926 the writer had the opportunity of staying on a farm in the Northern districts of Natal, South Africa. This farm—about 1,000 acres in extent and situated some 4,000 feet above sea level—is used primarily for sheep grazing, although cattle, horses and goats also form part of the farm stock. Half of the farm consists of a mountain rising to just above 5,000 feet above sea level, and the rest of the farm consists of an undulating plain. Except for a few depressions towards the foot of the mountain, which, during the rainy season become somewhat swampy, the whole farm may be considered as being very well drained. In consequence it is entirely covered with grass, there being no evidence of bush formation. Three rivulets, giving an abundant water supply, take their origin from the mountain.

During the writer's stay he made use of the opportunities offered to make a zoological survey of the helminth parasites occurring among the domestic and wild animals found on the farm. He was particularly struck with the paucity of helminth parasites in the wild animals, whereas not a single domestic animal examined was found to be free from parasites. The domestic animals examined were for the most part sheep, and consisted entirely of animals slaughtered for household use. The results would therefore tend to show the normal infection

found in apparently healthy animals in good slaughter condition. During this period 19 sheep, 3 lambs (5 months old), 2 calves, 1 cat, a few fowls, about 30 turtle doves (*Turtur capensis*), 1 rock pigeon, 3 black crows, 1 hammer head (*Scopus umbretta*), 5 butcher birds (*Lanius collaris*), 2 sand pipers (*Aegialites tricollaris*), about three dozen clawed toads (*Xenopus lævis*), and 3 *Rana* sp. were examined.

The types of the new species described below are to be deposited in the helminthological collections of the London School of Hygiene and Tropical Medicine and Veterinary Research Laboratories, Onderstepoort, Pretoria.

From *SHEEP*.

CESTODA.

Family ANOPLOCEPHALIDÆ.

Sub-family AVITELLINÆ.

STILESIA HEPATICA Wollfh., 1903.

This liver cestode was found, except in the lambs, in the gall bladder and bile ducts of all the sheep examined. As pointed out by Gouch, this parasite apparently produces no harmful effects on its host.

AVITELLINA CENTRIPUNCTATA (Rivolta, 1874) Gough, 1911.

Two sheep, one of which was a lamb, were found to harbour this parasite; in both cases, however, only a few worms were present.

Family TÆNIIDÆ.

Sub-family TÆNIINÆ.

ECHINOCOCCUS GRANULOSUS (Batsch., 1786) Rud., 1805.

Only a single case of Hydatid was found. This consisted of a single calcified cyst embedded in the lung.

TREMATODA.

Family PARAMPHISTOMIDÆ.

Sub-family PARAMPHISTOMINÆ.

COTYLOPHORON COTYLOPHORON (Fisch., 1901) Stiles & Goldberger, 1910.

This was the only trematode parasite found and was encountered on three occasions in the rumen of adult sheep. On one occasion more than 200 parasites were collected.

As far as the writer is aware this is the first time that this parasite has been recorded from South African sheep ; it is, however, a common parasite of cattle and various antelopes. Stiles and Goldberger (1910) described a species of this genus (*C. indicum*) from Indian sheep. Maplestone (1923), in his revision of the Amphistomes of mammals, considers that this species represents immature forms of *C. cotylophoron*. The writer is also inclined to this view as his specimens combine the characters mentioned by Stiles and Goldberger as distinguishing these two species. As Maplestone has correctly pointed out the nature of the œsophageal musculature, the position of the genital pore, and the nature of the genital atrium appear to be capable of a fair amount of variation, these variations being noted by the writer also in the mature specimens at his disposal.

NEMATODA.

Family TRICHOSTRONGYLIDÆ.

Sub-family TRICHOSTRONGYLINÆ.

HÆMONCHUS CONTORTUS (Rud., 1803) Stiles & Hass., 1905.

This is a very prevalent parasite and was found to be present in about half the adult sheep and in all the lambs. Each of the lambs, although in very good condition, harboured hundreds of these worms in the fourth stomach.

TRICHOSTRONGYLUS EXTENUATUS (Rail., 1898) Rans., 1907.

This very small parasite of the fourth stomach, together with the following, are notorious in South Africa for the amount of losses which they have caused. Popularly they are known as Bankrot Wurms (Bankruptcy worms) and well do they deserve their name. The writer was able to collect this parasite on only two occasions and then only in very small numbers.

TRICHOSTRONGYLUS INSTABILIS (Rail., 1893) Looss, 1905.

From the duodenum this parasite was collected on three occasions, twice in the host harbouring the previous species. Only a few worms were obtained.

Family ANCYLOSTOMIDÆ.

Sub-family NECATORINÆ.

MONODONTUS TRIGONOCEPHALUS (Rud., 1808) Rail., 1900.

This hookworm was obtained from an adult sheep on only one occasion. About 20 specimens were collected.

Family STRONGYLIDÆ.

Sub-family ŒSOPHAGOSTOMINÆ.

ŒSOPHAGOSTOMUM COLUMBIANUM Curtice, 1890.

This causative agent of pimply-gut was found to be present in all the adult sheep examined. As a rule although numerous nodules were present only a few parasites were collected, usually not more than a dozen from each sheep. Considering the large number of calcified nodules present and the good condition in which the sheep were, one begins to doubt whether the harmful effects of this parasite are really so serious as one is generally led to believe.

Family SPIRURIDÆ.

Sub-family GONGYLONEMINÆ.

GONGYLONEMA SCUTATUM (Leuck., 1873) Rail., 1892.

This parasite was encountered in the œsophagus of every adult sheep examined; usually there were about half a dozen worms present, but on one occasion 13 worms were obtained from one sheep.

GONGYLONEMA MONNIGI Baylis, 1926.

The writer found this parasite to be as common in sheep as the preceding; they were also absent from the lambs. The easiest method of collecting them was found to be that in which the whole stomach, after rinsing in cold water, was immersed into boiling water for about half a minute; the effects of this treatment was that the mucous lining peeled off very easily thus liberating the worms. The parasites, which the warm water had killed *in situ*, were found to keep very well when immediately transferred to luke-warm alcohol-glycerine.

As Baylis' description of the male is based on a single male, the writer gives the following data which is based on the examination of eleven specimens.

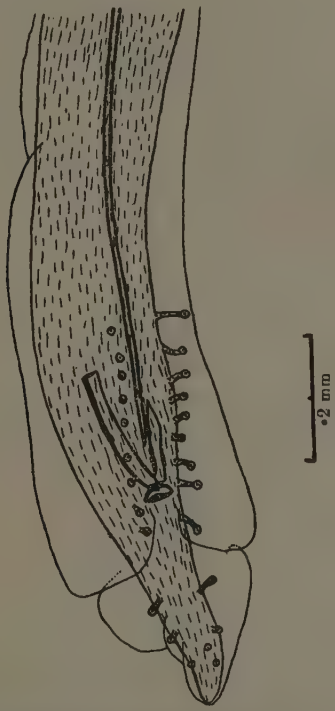


Fig. 1.—Gongylonema monnigi. Ventral view of tail of male.

The total length of the male in the writer's specimens was found to vary from 39 mm. to 44 mm., and the maximum thickness varied from 0·213 mm. to 0·262 mm. The cuticular bosses are confined to the left side, and the single cervical ala is very narrow and straight.

The mouth is surrounded by a cuticular rim from 0·026 mm. to 0·029 mm. in diameter and 0·007 mm. in height. No circum-oral

papillæ were observed although such structures have been reported from related species. The mouth is followed by an elongate buccal cavity or pharynx, which is from 0·035 mm. to 0·04 mm. in length by about 0·007 mm. in diameter. The œsophagus which follows is straight and consists of the usual two parts; the first and muscular portion varies in length from 0·46 mm. to 0·52 mm., and the second and glandular portion, which is about twice the thickness of the preceding portion, from 7·5 mm. to 7·8 mm. in length, *i.e.*, the total length of the œsophagus occupies about one-fifth of the body length. The nerve ring encircles the muscular portion of the œsophagus at about its middle, *i.e.*, it is found about 0·03 mm. from the anterior end. The excretory pore varies slightly in position according to the size of the worm, ; it was found from 0·46 mm. to 0·49 mm. from the anterior end.

The tail of the male is slightly twisted and is provided with two well developed cuticular expansions; these alæ are very much inflated and that of the left side is longer than that of the right side. In all the writer's specimens, except one, each ala is divided into two parts by a transverse constriction at about the junction of the first and second tail thirds. In Baylis' account no mention is made of these alar inflations or of their constrictions, which characters may possibly be due to the fact that the writer's specimens were killed with hot water. The posterior portion of the left ala generally passes round the tip of the tail towards the right side; this, however, is not always the case as in two specimens it was the right ala which passed round towards the left side. The number of caudal papillæ was found to vary; the largest number seen by the writer on one side is 13, of which 9 were pre-anal and 8 were limited to the anterior portion of the ala. The typical number appears to be 12 pairs, of which 8 pairs support the anterior portion of each ala and 4 pairs the posterior portion. All the papillæ, except the posterior 2 pairs, are pedunculate; these latter 2 pairs may be either pedunculate or sessile. The number of papillæ on one side may also not be equal to the number on the other side. The spicules are very unequal in size. That of the right side is short and stout and varies in length from 0·213 mm. to 0·25 mm. That of the left side is long and slender and ends in a chisel-shaped point; it was found to vary in length from 11 mm. to 15·7 mm. A gubernaculum is present varying in length from 0·11 mm. to 0·13 mm.

This species, as Baylis has pointed out, is very closely related to *Gongylonema verrucosum* (Giles), the chief points of difference being that in the latter there is a broad and festooned cervical ala on the left side, and the gubernaculum is shorter and broader and of different shape.

Family TRICHINELLIDÆ.

Sub-family TRICHURINÆ.

TRICHURIS OVIS (Abildg., 1795) Smith, 1908.

This very common parasite of English sheep was obtained on only one occasion and then only a single specimen was collected.

From *CATTLE*.

CESTODA.

Family ANOPLOCEPHALIDÆ.

Sub-family ANOPLOCEPHALINÆ.

MONIEZIA sp.

Some fragments of a partially decomposed Moniezia were collected from the fæces of a calf after treatment with a mixture of arsenic and copper sulphate.

NEMATODA.

Family ASCARIDÆ.

Sub-family ASCARINÆ.

ASCARIS VITULORUM Gœze, 1782.

A few male and female specimens were passed by a calf after treatment with pure copper sulphate.

From *CAT*.

NEMATODA.

Family ANCYLOSTOMIDÆ.

Sub-family ANCYLOSTOMINÆ.

ANCYLOSTOMA CANINUM (Erc., 1859) Hall, 1913.

Seven specimens were recovered from the small intestine of a domestic cat.

From *DOMESTIC FOWLS.*

NEMATODA.

Family HETERAKIDÆ.

Sub-family HETERAKINÆ.

HETERAKIS PAPILLOSA (Bloch, 1782) Rail., 1885.

This parasite was found to be present in the cæca of all the fowls examined.

ASCARIDIA LINEATA (Schn. 1866) Rail. & Henry, 1912.

Two specimens were on one occasion collected from the small intestine.

Family SPIRURIDÆ.

Sub-family GONGYLONEMINÆ.

GONGYLONEMA INGLUVICOLA Rans., 1904.

A few female examples of this parasite were on one occasion collected from the crop of a fowl. As no male were present they are only tentatively referred to the above species.

Sub-family ACUARIINÆ.

ACUARIA (ACUARIA) sp.

On one occasion a single female specimen of this parasite was found embedded in the gizzard lining.

From *WILD ANIMALS.*

CESTODA.

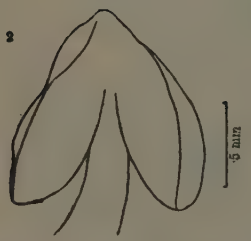
From all the wild animals examined only two species of Cestodes were collected, viz.: one specimen of the anoplocephalid cestode, BERTIELLA DELAFONDIA (Rail., 1892) from the Cape Turtle Dove (*Turtur capensis*), and a new species of bothriocephalid cestode from the clawed toad.

Family DIBOTHRIOCEPHALIDÆ.

DIBOTHRIOCEPHALUS XENOPI n. sp.

This small cestode was obtained on two occasions from the small intestine of the clawed toad (*Xenopus lævis*); only a few mature specimens were collected on each occasion.

The total length of the worm reaches a maximum of 16 mm., with a maximum breadth of 1·6 mm. across the last segment. The scolex is large and carries two large bothridial grooves whose posterior margins stand away from the strobila and thus give the head an arrow-shaped appearance ; they are from one to 1·2 mm. in length with a maximum breadth across their middle of about 1 mm. ; they are dorsal and ventral in position and have their free lateral margins slightly inrolled. The whole head thus has an appearance very similar to that found in *Polyonchobothrium belones* (= *Ptychobothrium belones*). The strobila, which at its anterior end has a breadth of only about 0·6 mm., is flattened dorso-ventrally and increases gradually in breadth towards



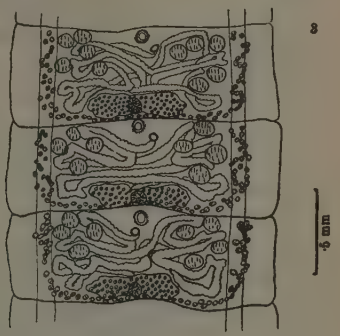


Fig. 2.—Dibothriocephalus xenopi. Lateral view of scolex.
Fig. 3.—Dibothriocephalus xenopi. Three mature segments from the ventral side, showing the positions of the genital organs.

its posterior extremity where it attains its greatest width. Its most anterior portion may be regarded as forming a neck, there being no indication of external or internal segmentation in the first mm. of its length. After this the internal genitalia are seen to make their appearance followed soon after by traces of external segmentation. At about 5 mm. from the anterior end the internal genitalia are fully developed and immediately after eggs are seen to be present in the uteri. In the posterior half of the strobila external segmentation can be made out with the naked eye. The segments, which are throughout much broader than long, are at first very short, but their length increases towards the posterior end, where they may reach a length of nearly 0·5 mm.

The internal genitalia are arranged in a manner more or less typical for the genus. The genital cloaca is situated in a slight elevation of the cuticle in the mid-line of the ventral surface towards the anterior margin of the segment. Into it both the vas deferens and vagina open by a common aperture. On the same surface, slightly posterior of the genital cloaca, there is situated the uterine pore ; as a rule its situation is slightly to the left of the mid-line, but it may occupy a corresponding position on the right side.

The ovary is situated towards the base of the segment and consists of two imperfectly separated wings, the two wings being joined to each other by two bridges of ovarian tissue. The space between these bridges is occupied by the shell gland, the bridges passing respectively anterior and posterior to it. The ovarian elements are very loosely packed together. The oviduct arises from the posterior face of the anterior ovarian bridge and passes dorsalwards through the shell gland ; before entering this organ it receives the opening of the common vitelline duct. After emerging from the shell gland it passes forwards to join the uterus which winds to right and to left of the segment in between the testes ; its coils, however, do not pass laterally beyond the longtitudinal excretory vessels. It is distended with eggs which are discharged to the exterior through the uterine pore. The eggs are oval and thin-shelled, and, from the preserved material examined, appear to be non-operculate ; they measure 0·037 mm. long by 0·026 mm. in breadth.

The vagina is a thin-walled tube which passes inwards and backwards from the genital cloaca ; just anterior to the ovary it is dilated to form a spacious receptaculum seminis. The junction between it and the oviduct was unfortunately not observed.

The vitellaria consist of two lateral bands of follicles immediately ventral and parallel to the longtitudinal excretory vessels ; towards the posterior margin of the segment they pass inwards behind the posterior margin of the ovary. Here the two short vitelline ducts meet immediately ventral to the shell gland, and the common duct passes forwards and upwards to meet the oviduct prior to its entry into the shell gland.

The testes are very striking, being relatively few in number and large. The largest number seen in one segment was six on either side, and the

smallest was three on one side and four on the other; generally the number of testes on each side is equal, the groups being separated by the median uterine coils. The testes are oval and each extends from the dorsal to the ventral layer of longtitudinal muscles; they are from 0·1 mm. to 0·15 mm. long with a diameter of from 0·066 mm. to 0·082 mm. The vasa efferentia were not followed but the distal end of the vas deferens is enlarged to form a vesicula seminalis which, after forming a few loops, passes ventralwards towards the genital cloaca.

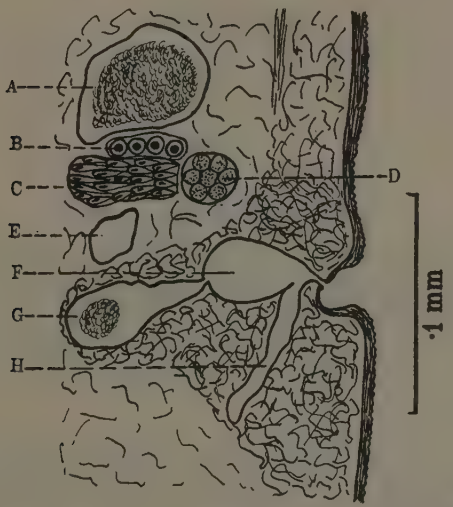


Fig. 4.—Dibothriocephalus xenopi. Vertical longtitudinal section passing through the genital cloaca. A = Receptaculum seminis; B = Ovary; C = Shell gland; D = Common Vitelline duct; E = Section of transverse Excretory vessel; F = Vesicula seminalis externa; G = Vesicula seminalis; H = Vagina.

In the denser parenchymatous tissue surrounding the genital cloaca it gradually narrows to finally open into a pear-shaped cavity (vesicula seminalis externa ?) which receives the opening of the vagina and then opens into the base of the genital cloaca by a very short hermaphrodite duct.

The excretory system is represented by two large longitudinal vessels, one on either side of the entire strobila ; each has a diameter of about 0·06 mm., and the two are connected with each other by a transverse duct, about 0·035 mm. in diameter, which passes along the posterior margin of the segment, immediately posterior to the ovary and dorsal to the incurving bands of vitelline glands.

The chief distinguishing features of this cestode are its small size, the number of its testes which are few and large, and size and shape of its scolex.

TREMATODA.

Only one species of fluke was obtained and it proved to be a new species.

Family PARAMPHISTOMIDÆ.

Sub-family DIPLODISCINÆ.

DIPLODISCUS DOYERI n. sp.

This parasite was collected on three occasions from the rectum of the clawed toad (*Xenopus lævis*). Eight mature specimens were obtained. The writer has much pleasure in naming this species after Mr. O. Doyer of Babanango, Natal, in recognition of the friendly interest he took in the work and the assistance he rendered the writer on several occasions.

In the specimens which were killed according to Looss' method in hot Schaudinn's solution, the body is round and cone-shaped, and varies in length from 2·3 mm. to 3 mm. ; its greatest thickness is just anterior to the acetabulum where it is from 0·5 mm. to 0·6 mm. in diameter. The oral sucker is terminal in position and its opening is directed forwards ; towards its anterior border it is provided with a layer of circular muscles, which serve to open or close its external orifice ; the rest of the sucker is provided with the usual muscles radiating outwards from its lumen. Two oral pouches take their origin from the postero-lateral angles of the oral sucker ; these are provided with the same type of musculature as the sucker, and are directed obliquely away from it towards the lateral margins. The acetabulum is relatively very large, being from 1·1 mm. to 1·4 mm. in diameter ; it is directed postero-ventrally and its inner

surface is raised to form a number of irregular ridges or flanges which tend to take a circular but wavy course.

The mouth is situated at the base of the oral sucker ; it leads into a long pre-pharynx which emerges from the sucker between and slightly ventral of the pouches, and then proceeds obliquely backwards and dorsalwards to join the pharynx. The pharynx is an oval and muscular organ which is joined to the intestinal cæca by a very short œsophagus. The cæca are short and club-shaped, and only extend about halfway between the level of the pharynx and that of the excretory pore.

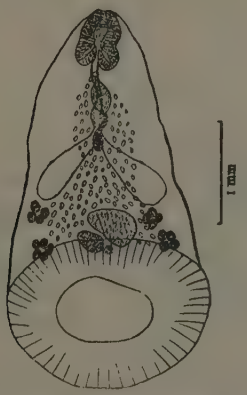


Fig. 5.—Diplodiscus doyeri. Ventral view of flattened specimen.

The genital cloaca is fairly large and is very anteriorly placed being situated just posterior of the level of the hind border of the oral pouches, and some distance in front of the bifurcal zone. At its base there is a small genital cone on the tip of which the hermaphrodite duct opens.

The male genital system consists of a single large and somewhat oval testis situated in the mid-line of the body. The vasa efferentia unite near the origin of the intestinal cæca and the resulting common duct is dilated to form a conspicuous vesicula seminalis which follows a slightly wavy course to join the pars prostatica ; this structure is oval in shape and about 0·1 mm. long by about 0·07 mm. in thickness.

The ductus ejaculatoris which follows is short and soon receives the opening of the uterus ; the common or hermaphrodite duct thus formed passes ventralwards towards the genital cloaca to open to the exterior on the apex of the genital papilla.

The single ovary is oval and only about one-third the size of the testis. It is situated between the testis and the acetabulum. The oviduct arises from its dorsal surface. The shell gland is small and is situated postero-dorsal of the ovary. The uterus is voluminous and passes from one side to the other dorsal of the testis and ventral of the intestinal cæca ; at about the level of the pars prostatica it becomes muscular

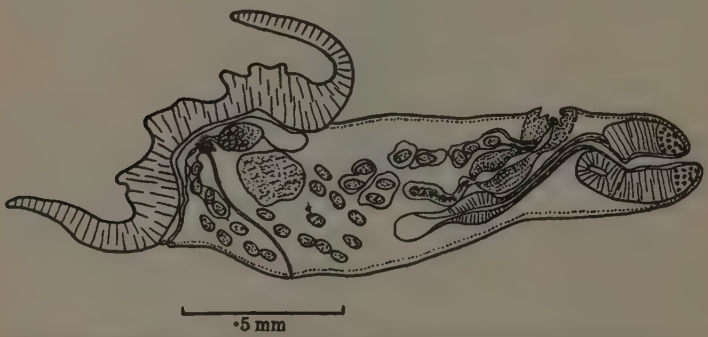


Fig. 6.—Diplodiscus doyeri. Vertical longtitudinal section, reconstructed from several serial sections.

and then joins on to the ductus ejaculatoris to form the hermaphrodite duct as already mentioned. A Laurer's canal is present ; it passes obliquely upwards and forwards to open in the dorsal mid-line at about the junction of the third and last body quarters. The vitelline glands consist of a few groups of large follicles on each side of the body, and are situated ventral of the ovary and posterior of the intestinal cæca ; their two collecting ducts meet ventral of the shell gland and the common duct discharges into the oviduct prior to the latter's entry into the shell gland.

The excretory system is of the usual type found in this genus. The excretory pore is in the dorsal mid-line immediately anterior of the acetabulum. It leads into an elongate excretory vesicle which passes ventralwards following the anterior margin of the acetabulum. From its ventral termination two tubes pass forwards and dorsalwards, making a few irregular loops along the outer border of the intestinal cæca. The whole of the internal surface of the excretory tubes and vesicle is lined by dark and granular concretions.

Only five members of the genus Diplodiscus have been previously described, viz.: *D. subclavatus* (Pallas, 1760) from Europe; *D. temperatus* (Staff., 1905) and *D. americanus* (Chandler, 1923) from America; and *D. megalochrus* (John., 1912) and *D. microchrus* (John., 1912) from Australia. The above described species is therefore the first record from South Africa, and it differs from the other five species in the very anterior position of its genital cloaca. The large size of its acetabulum and its limited number of vitelline glands allies it to *D. americanus*, from which species, however, it is easily distinguished in that the former has two testes.

NEMATODA.

Nematodes were collected on only two occasions; once a male and a female of ASCARIDIA COLUMBÆ (Gmel., 1790) Trav., 1913, were obtained from a rock pigeon, *Columba phœonota*, and once a number of larval spirurids were collected from the stomach of a hammer head (*Scopus umbretta*).

ACKNOWLEDGMENT.

The writer wishes here to express his indebtedness and thanks to Sir Arnold Theiler, K.C.M.G., Director of Veterinary Education and Research, Pretoria, for his kindness in placing at the writer's disposal a microscope and apparatus necessary for the collection of the above listed helminths.

REFERENCES.

ARIOLA, A., 1900.—"Revisione dell famiglia Bothriocephalidæ s. str." *Arch. de Parasit.*, vol. III., pp. 369-484. Paris.

BAER, J. G., 1925.—"Contributions to the Helminth-Fauna of South Africa." Thesis, Univ., Neuchatel. Pretoria.

BAYLIS, H. A., 1925.—" On the species of Gongylonema (Nematoda) parasitic in Ruminants." *Jnl. Comp. Path. & Therap.*, vol. XXXVIII., pp. 46-55. Edinburgh.

BAYLIS, H. A., 1926.—" On the Male of *Gongylonema verrucosum* (Giles) (Nematoda) and on a new species of Gongylonema from the Sheep." *Ibid.*, vol. XXXIX., pp. 134-137.

BRAUN, M., 1896.—" Cestodes." *Bronn's Klassen und Ordnungen des Thier-Reichs*, vol. IV., Part 1B. Leipzig.

CHANDLER, A. C., 1923.—" Three new Trematodes from *Amphiuma means*." *Proc. U.S. Nat. Mus.*, vol. LXIII., pp. 1-7. Washington.

GOUGH, L. H., 1911.—" A Monograph of the Tapeworms of the Sub-family Avitellininæ, being a Revision of the Genus Stilesia, and an Account of the Histology of *Avitellina centripunctata* (Riv.). *Quart. Jnl. Micros. Sci.*, vol. LVI., pp. 387-385. London.

JOHNSTON, S. J., 1912.—" On some Trematodes—Parasites of Australian Frogs." *Proc. Linn. Soc. New South Wales*, vol. XXXVII., pp. 302-308. Sydney.

LUEHE, M., 1899.—" Zur Anatomie und Systematik der Bothriocephaliden." *Verhandl. Deutsch. Zool. Gesell.*, pp. 30-55.

LUEHE, M., 1909.—" Parasitische Plattwurmer. I., Trematodes," in *Die Suss-wasserfauna Deutsch.*, Part 17, pp. 35-40. Jena.

LUEHE, M., 1910.—" Parasitische Plattwurmer. II., Cestodes." *Ibid.*, Part 18, pp. 13-28.

MAPLESTONE, P. A., 1923.—" A Revision of the Amphistomata of Mammals." *Ann. Trop. Med. & Parasit.*, vol. XVII., pp. 113-105. Liverpool.

NYBELIN, O., 1922.—" Anatomisch-Systematische Studien uber Pseudophyllideen." *Goteborg's Kungl. Vetensh.-och. Vitt. Sam. Handl.*, vol. XXVI., pp. 1-228. Gotenberg.

RANSOM, B. H., 1904.—" A New Nematode (*Gongylonema ingluvicola*) parasitic in the Crop of Chickens."

RANSOM, B. H., 1911.—" The Nematodes parasitic in the Alimentary Tract of Cattle, Sheep, and other Ruminants." *U.S. Dept. of Agric. Bur. Anim. Indust.*, Bull. 127, pp. 1-132. Washington.

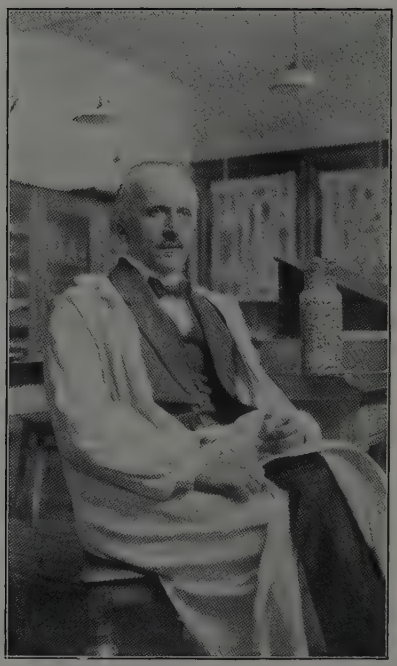
SCHWARTZ, B., 1925.—" *Ascaridia lineata*, a parasite of Chickens in the United States. *Jnl. Agr. Res.*, vol. XXX., pp. 763-772. Washington.

STAFFORD, J., 1905.—" Trematodes from Canadian Vertebrates." *Zool. Anz.*, vol. XXVIII., pp. 689-690.

STILES, C. W. and GOLDBERGER, J., 1910.—" A study of the Anatomy of *Watsonius* (n.g.) *watsoni* of Man and of nineteen allied Species, etc." *Hyg. Laboratory Bull.*, 60, pp. 60-73. Washington.

STUNKARD, H. W., 192–.—" The present Status of the Amphistome Problem. *Parasitology*, vol. XVII., pp. 137-148. Cambridge.

VOLZ, W., 1900.—" Beitrag zur Kenntniss einiger Vogelcestoden." *Arch. f. Naturg.*, vol. LXVI., pp. 165-170. Berlin.



PROFESSOR FUHRMANN, Neuchatel.

Journal of Helminthology, Vol. IV., *Nos.* 4-5, *Nov.*, 1926; *pp.* 143-178.

Serological Studies on Hydatid.

By I. Hiles, M.Sc.

Research Assistant to the Professor of Helminthology in the University of London.

(*From the Helminthological Department, London School of Hygiene and Tropical Medicine.*)

The Bordet-Gengou test applied to the diagnosis of cases of suspected hydatid infection has been used by many workers since first introduced by Ghedini in 1906, using as antigen hydatid fluid from human cysts.

He was followed by Apphatie and Lorenz, 1908, Weinberg, 1908, Weinberg and Parvu, 1908, Weinberg and Vieillard, 1909, Bettencourt, 1909, Jianu, 1909, Israel, 1910, Henius, 1911, and others.

Braunstein, 1910, said the serum in cases of hydatid infection had a certain anti-complementary property.

Dobrotine, 1910, advocated the use of human cyst fluid, whereas Weinberg, 1909, Putzu, 1909, Abrikosow, 1913, and others used sheep fluid saying that fluid from human sources might give a positive reaction with normal sera.

Kreuter, 1909, Durand, 1909, supported Dobrotine.

Other workers again—Vas, 1911, Barsony and Egan, 1912, among others—used fluid from cattle cysts; from these workers Putzu, 1910, differed as he found he got a positive result with some normal sera when using fluid from cattle cysts.

Graetz, 1910, employed fluid from pig cysts and got good results whereas Kitzler did not succeed with it.

Weinberg, 1913, advised fluid from sheep cysts rather than from human, ox, pig, horse or camel cysts.

Many of these workers found that the test did not always succeed, *i.e.* that sera from undoubted cases of infection might not react positively.

Other workers in the early days included Faure, 1909, Routier, 1909, Delbet, 1909, Walther, 1909, Rossello, 1909, de Gaetano, 1909, Puntoni, 1910, Lejars, 1910, and others.

Sonntag, 1913, commented on the great differences in fluids and said that they must always be standardised.

Dobrotine, 1910, testing a human case with *Echinococcus multilocularis* got a positive reaction using fluid from a human " simple " cyst.

Zapelloni and Ricciuti, 1910, using hydatid fluid in doses varying from ·2–·6 c.c., got a positive reaction with 23 sera out of 39 tested.

Thomsen and Magnussen, 1912, stated that titration of the fluid was necessary as, whereas in some cases a dose of ·05–·1 c.c. was sufficient, with others 1–2 c.c. was required.

Animal Infections.

Some of the workers applied the test to animal infections with varying results.

Weinberg and Parvu, 1908, got 3 positive results in 5 cases of cattle tested, whereas Puntoni, 1910, got 4 strong, 5 feeble and 8 negative reactions out of 21 tested; he also got weakly positive results with 5 syphilitic sera, 1 case of tuberculosis and with 2 sheep harbouring Strongyles.

Weinberg and Parvu tested sheep also and got some good results, Weinberg following this up further but not always being successful; he and Vieillard, 1909, got a positive reaction in one case of horse and one of camel infection.

Graetz, 1910, got positive reactions with 4 pigs; he showed that rabbit sera may give a non-specific reaction for hydatid.

Other Antigens Employed.

Treated hydatid fluid as antigen.

Some workers were not satisfied with pure hydatid fluid as antigen and tried various modifications.

Parvu, 1909, treated the fluid with alcohol in the proportions of 1 volume of fluid to 5 volumes of alcohol, got rid of the precipitate, evaporated down the fluid at 60° c. and took up the residue in saline.

Jianu, 1909, and Stenza, 1909, used an ether extract of hydatid fluid, as did Falcoiano in 1913.

Kreuter, 1911, evaporated down some fluid and re-dissolved the residue in physiological saline. He also prepared an alcoholic extract of the residue.

Extracts of membrane as antigen.

Braunstein, 1910, stated that he found watery extracts of cyst membrane equally useful with hydatid fluid. Israel, 1911, evaporated down an aqueous extract of membrane and from the residue made an alcoholic extract. Henius, 1911, used aqueous extract of membrane and Hahn, 1912, stated that hydatid fluid was not sufficiently antigenic and used an aqueous extract also. Kreuter, 1911, denied the usefulness of aqueous extracts and prepared alcoholic extracts instead. He found the complement fixation test of no great value diagnostically, at the most giving a positive reaction in 50 per cent. of the cases. He pointed out that if used in large amounts the extracts might give pseudo-positive reactions with syphilitic, leprosy or even normal sera—a danger that could be obviated by using smaller doses. Israel, 1911, Busson, 1911, Vas, 1911, and others also reported on the tendency of alcoholic extracts to give deceptive results.

Urioste and Scaltritti, 1911, found hydatid fluids very variable, one out of five might prove useful. They got 8 positive reactions out of 17 sera tested. They stated that hydatid fluid run through a Chamberland filter loses part of its antigenic property. They passed about 2 litres through, dried the candle and treated it with 150 c.c. of ether; they evaporated down the ethereal solution, mixed the dried residue with ·5–1 gramme of dry, finely powdered salt and kept this as a stock mixture. For use they dissolved ·09 gramme of this in 10 c.c. of distilled water. They also evaporated some hydatid fluid and treated the residue with ether.

A "Group" Reaction.

Kurt Meyer, in 1911, tested the sera of some carriers of *T. saginata* with hydatid fluid and also with watery and alcoholic extracts of membranes and got positive results. He also reversed the action, making *Tænia* extracts and getting positive results with sera from hydatid cases. He concluded that this pointed to the reaction being a "group" rather than a specific one. He was supported in this by Kreuter, 1911, Hahn, 1912, Pfeiler, 1912, and Barsony and Egan, 1912.

On the other hand, Thomsen and Magnussen, 1912, and Sonntag, 1913, denied the interaction of *Tænia* and hydatid.

Weinberg, in Kolle and Wassermann's "Handbuch der pathogenen Mikro-organismen," in 1913, dealt with the whole question of complement-fixation test for hydatid infection, and summarised the results of previous workers as follows :—

"Of 306 cases tested, in 17·97 per cent. the test failed." In another series of 60 of his own 11 failed and 3 gave very feeble reactions.

Zapelloni, in 1915, again collected statistics from literary evidence. Out of 535 cases the test failed in 12 per cent. ; he himself got positive reactions with serum from *E. multilocularis* as well as the usual *E. hydatidosus*. He found the meiostahmin and precipitin tests useless and stated that suppuration and degeneration of the cyst may lead to the disappearance of antibodies in the blood, whereas Parvu, 1912, found that suppuration may intensify the reaction. Weinberg, 1913, stated that in cases of suppuration the reaction may be stronger, as Apphatie and Lorenz found was the case with 6 sera tested by them.

Pontano, 1920, disagreed, but Fairley, 1922, found the test in such cases to give positive results.

More Recent Work.

Dubot, in 1919, tested many fluids and found great variations in antigenic strength ; he found no anti-complementary action with fluids of animal origin, in that point differing from Weinberg, who found it present in some fluids. Dubot used constant volumes of serum and hydatid fluid and varied the complement. He did a series of serum and antigen controls with the same doses of complement and read the

results as positive if, in the test proper, more units of complement were deviated than with serum and antigen separately.

Pewny, 1921, stated that the cyst membrane could yield Wassermann-antigenic properties.

Rubinstein, 1921, used pure hydatid fluid, varying the volume of sheep's corpuscles using 3 tubes, setting up the serum control in the same way, thus demonstrating the anti-complementary power of the serum alone and of the serum together with antigen.

Hoffmann (quoted by Rabinowitsch) said that many animal bloods (ox, buffalo, sheep) show active anti-complementary properties.

Blumenthal, 1921, emphasised the importance of careful titration beforehand of the amboceptor with the complement and antigen and gave his method. As antigen he used a hydatid fluid kept in the dark at room-temperature for at least three months, with the addition of ·5 per cent. of the volume of 10 per cent. phenol. He advocated as dose the highest dilution which gave negative results with normal and with syphilitic sera and a strongly positive one with echinococcal serum. This usually worked out at about ·1 c.c. of fluid with ·15 c.c. of isotonic saline. He and Unger, 1923, worked together on the same lines. They found that fluids varied greatly, and used three or four different ones for each test (one at least of these to be of animal origin), and at least two must give a strongly positive reaction. They quoted one case operated on in 1916, which developed another cyst in the same locality in 1922: the test was negative before the second operation.

Fairley, 1922, advocated the use of undiluted clear hydatid fluid (from healthy cysts containing scolices) which, as it never showed hæmolytic tendencies, need not be titrated. He advised against using any antiseptic but to draw off the fluid with sterile precautions and to keep on ice.

Other antigens he suggested were saline and alcoholic extracts of well-washed scolices, but these needed careful standardisation as, if used in concentrated solutions, they may act as weak syphilitic antigens.

He advocated fluid from sheep rather than from human cysts. He found extracts of cyst membrane useless and suggested that the antigenic property was derived from the scolices or the product of their

activities. He stated that suppuration of the cyst caused failure of the intradermal test but not of the precipitin test nor of the complement-fixation reaction.

Rabinowitsch, 1923, made a saline extract of hydatid fluid and cyst wall, going through a lengthy process at different temperatures. He prepared four batches of this, of which two only proved successful. He found that ·05 c.c. was strongly anti-complementary and gave a partial reaction with normal, and a stronger one with syphilitic serum ; by reducing the amount to ·03 c.c. he got a reaction with hydatid serum only, so in the test used ·03, ·02 and ·01 c.c. He advocated using both heated and unheated serum and got 59 per cent. strongly positive both ways, 12·8 per cent. strongly positive with inactivated and weak with unheated sera, 23·1 per cent. weak with inactivated and strong with unheated sera, and 5·1 per cent. giving the reaction exclusively with one or the other.

Van der Hoeden, 1923, said that the antigenic properties depended on the scolex-content of the cyst—supporting Fairley—and not on the amount of albumin present. He denied the usefulness of aqueous and alcoholic extracts of cyst wall and of mother and daughter cysts.

Thiellé, 1923, used sheep, ox, and human cyst fluids, and got approximately 50 per cent. positive with sheep, 45 per cent. with ox (20 cases), 62·5 per cent. with pig (8 cases), and 48 per cent. with human cyst fluid (23 cases), and concluded that human and pig cyst fluids were the more active, the fluid to be clear and unheated. Both the rapid and slow methods of Weinberg were employed, giving a larger number of positive reactions (more or less strong) with the former, in which the serum was unheated. No guinea pig nor hæmolytic serum was used and larger volumes of fluid (·2–·4 c.c.) were employed. In the slow (usual) method ·1 c.c. of fluid diluted one-half or one-third was the dose used.

Patterson and Williams, 1923, used alcoholic extracts, evaporating down the requisite volume at room temperature and taking up the residue in distilled water, diluting one-third with saline ; this was in order to avoid pseudo-positive reactions with syphilitic sera. In 30 cases tested 25 gave complement-fixation with hydatid fluid and all 30 with extract. I understand that further experience with this form of extract has not quite realised expectation.

Bryce, Kellaway and Williams, 1924, quoted a case in which they used both hydatid fluid and alcoholic extract, and found that with the former the serum fixed 2–3 M.H.D. of complement, whereas with the latter four or more M.H.D. were fixed. In 129 positive cases, 44 gave the same results with both antigens, 45 gave a more marked reaction with alcoholic extract, 8 more marked with hydatid fluid, 17 reacted only with extract, and 15 only with fluid. They found that some hydatid fluids had a distinct hæmolytic effect on sheep's corpuscles and this property was not altered by heating for one hour at 55° C.

They also tried tryptic digests of fresh scolices and of those extracted with alcohol. The former gave potent antigens in dilutions of 1 : 20, the latter were weak. They also made acid and alkaline hydrolysates, the latter proving fairly active but giving pseudo-positive reactions with syphilitic sera and even with negative ones. They did not succeed in preparing a dried antigen to be used quantitatively for all types.

Dew and Williams, 1924, used alcoholic extracts of scolices from sheep cysts and got 80 per cent. of positives; they also tried saline solution extracts of scolices digested with trypsin.

Horowitz-Wlassowa, 1926, used hydatid fluid from cysts of men and cattle and said a completely pellucid fluid seemed to have no antigenic power; but a cloudy one containing the products of disintegration of the scolices, cyst wall, etc., was active even in small doses. He advocated previous titration of the complement and testing of the antigen to be sure the working dose did not itself bind the complement. He got two failures in 20 sera tested.

As regards the nature of the antigen he stated that, as there are only traces of albumin present, it is apparently mainly of lipoidal nature; heating for one hour at 55° C. did not seem to lessen its antigenic property and he suggested it might be preserved by that method.

Comparison of the Value of the Different Biological Tests.

Pontano, 1920, came to the conclusion that, of the various biological tests, the Casoni (intradermal) gave 84 per cent. of positives, the subcutaneous 66 per cent., the complement-fixation test 50 per cent. and the eosinophile count showed an increase in only 40 per cent. of the cases examined.

Luridiana, 1921, said that the intradermal and complement-fixation tests were relatively the more reliable but that neither was always dependable and that their results did not always agree.

Ithurrat and Calcagno, 1922, found none of the tests to be infallible ; they placed most reliance on the intradermal test, getting only four results discordant with the findings at operation whereas the complement-fixation test gave only 70 per cent. correct. They used fluid from animal rather than human sources and stated that suppuration of the cyst did not affect the intradermal test.

Albo, 1922, discussing cysts in the pancreas, stated that in cases of hydatid infection " neither eosinophilia nor deviation of complement were normal findings."

Deluca, 1922, in discussing the intradermal test, found that hydatid fluid was a very feeble sensitiser and that cyst extracts were better.

Dew and Williams, 1924, found the intradermal test absolutely specific as an aid to diagnosis, but needs care in interpretation.

Mogena, 1924, got positive reactions in about 73 per cent. of the cases he tested and declared the intradermal test to be much better, the results of that test in 14 cases being all confirmed at operation.

Trenti, 1924, found the intradermal test successful in 85 per cent. of his cases, the subcutaneous next and the complement-fixation last in reliability, only 43 per cent. reacting positively.

Goudsmit, 1924, found that in two cases where the test was negative or feebly positive he got a good reaction with the intradermal test and advocated the latter as more reliable.

Jaffé, 1925, said the complement-fixation test was of doubtful value. He tested five cases and got four negative and one doubtfully positive reaction ; the four negatives on operation proved to have each a cyst varying from the size of a fist to that of a child's head ; he used hydatid fluid as antigen. He declared that the blood pictures and the results of the complement fixation test (using hydatid fluid) can be accepted only with the greatest care and after consideration of all associated symptoms.

Deusch, 1925, advocated the intradermal rather than the complement-fixation test which he found to fail rather more frequently than some of the other workers. Three cases of lung infection gave negative results and a fourth gave a doubtful one becoming positive after the intradermal test had been performed. He refers to the non-specific reaction with syphilis, leprosy, liver-carcinoma and ascaris infections.

From the foregoing résumé of the literature on the subject of the complement-fixation and other tests for hydatid infection it is evident that opinions have differed both as to the reliability of the tests and as to the form of antigen of the most value ; the consensus of recent opinion seems to lay greater stress on the intradermal rather than the complement-fixation test. This is not a laboratory but a consulting room test and rests in the hands of the physician rather than of the pathologist. It was thought worth while to try and obtain further evidence on the value of the complement-fixation reaction as a means of laboratory diagnosis and to investigate a little further the possibilities of various forms of antigen, with special reference to the preparation of a stable, concentrated form to serve as a stock solution and to require considerable dilution for use. Among workers in countries where the occurrence of hydatid infection is more common than it is in England, the use of pure undiluted hydatid fluid as antigen is advocated ; this fluid must be drawn off with sterile precautions from healthy active cysts, *i.e.* cysts containing a fair number of living scolices and not showing signs of degeneration ; the fluid also must be absolutely clear and is to be rejected if at all turbid. Owing to the much greater prevalence of the disease among the cattle and sheep of those countries, a supply of cysts can easily be obtained at any time at the slaughter-houses. In this country it is not possible to rely upon being able to obtain at short notice suitable cysts of this kind—or indeed cysts at all—on any day that they may happen to be required as, fortunately from the standpoint of the health of the beasts here, infection is not very common, indeed, days may go by without any being obtainable even at large abattoirs.

Again, of such as have been brought in, the fluids on being tried have in the great majority of cases failed to give good results, possibly because the scolex-content was not sufficiently high or that the elimination into the fluid of certain products of the activity of the scolices was not sufficiently marked. Most of the fluids tried came from cysts in horses, a few only being from sheep cysts. If a good fluid be obtained there is always the difficulty of preservation ; workers are generally agreed that the addition of phenol or formalin, even in small quantities, is not desirable in the case of a fluid to be used for so delicate a test as the complement-fixation one. To keep a supply for an indefinite period requires cold-storage facilities such as are not included in the equipment of all laboratories.

Alcoholic extracts of hydatid material have therefore been prepared in several forms in an endeavour to obtain a suitable antigen which, once standardised, can be kept for some period of time and be ready for use when required.

In order to prepare these alcoholic extracts in various ways a considerable supply of cysts was necessary; the scolices were found, as Fairley stated, to be the best material for extraction and in order to obtain any bulk of these a large amount of hydatid fluid had to be centrifugalised so that a considerable period of time was required to get the cysts collected and the extracts prepared in sufficient quantity. The cysts were obtained mainly from horse material with a certain proportion from sheep and pigs; from their contents the following forms of antigen were prepared:—

I.—*An absolute-alcohol extract of washed scolices.*

A large amount of hydatid fluid was gently centrifugalised and the deposit then well washed with saline and again centrifugalised; a second and third washing and centrifugalising followed; the saline was then pipetted off as far as possible and the deposit drained; the volume was noted and it was then scraped into a mortar and an equal volume of sand and sufficient absolute alcohol to moisten the mixture were added and the whole finely ground (the sand had previously been thoroughly washed with saline, dried and then washed with alcohol). The mixture was transferred to a small stoppered bottle, the mortar rinsed out with a little alcohol and sufficient more of the latter added to make a 10 per cent. or 20 per cent. suspension as might be required. The bottle, tightly stoppered, was kept at 37° C.—with frequent shakings—for from 2-7 days. Finally the alcohol was carefully decanted off or filtered through fat-free filter paper, absolute alcohol added to make up to the original volume and the solution stored in a well-stoppered bottle in the dark at room-temperature.

II.—*An absolute alcohol extract of washed and dried scolices.*

The method of preparation here was the same as above but after the thorough washing and centrifuging the moist scolices were transferred to a watch glass and dried over H_2SO_4 or $CaCl_2$; when dry the deposit was weighed and transferred to a mortar, and 20 per cent. extract made.

III.—*An absolute alcohol extract of acetone-treated scolices.*

Here the scolices, after washing and draining, were treated with four to five times their volume of acetone for one hour with frequent shaking when the acetone was poured off, the residue ground up, dried and then treated with absolute alcohol as above, making 20 per cent. extract.

IV.—*50, 70 and 90 per cent. alcoholic extracts of the same material.*

The procedure here was exactly the same as for No. II., but alcohols of the three strengths mentioned were used instead of absolute alcohol.

V.—*An absolute alcohol extract of cyst membrane.*

The cyst membranes were as far as possible scraped free of scolices, then cut up in small pieces and well washed three times with saline; after draining on filter paper they were weighed, transferred to a mortar and treated in the same way as the scolices for No. I.

The Time of Extraction.

Samples of material from the same batch of cysts were kept at 37° C., being shaken at frequent intervals, for periods varying from 3-7 days, the method of treatment being otherwise the same.

On standardisation, the extracts in each case gave practically the same titre, seeming to indicate that in three days at 37° C. the absolute alcohol extracts the antigenic property equally as well as in seven days. Alcoholic extracts of the various forms, kept in the dark at room-temperature, were practically as active 12 months later as when they were prepared.

One batch of extracts was made from the scolices collected from some cysts which had been placed in 5 per cent. formalin for two or three days; this extract was found to be practically devoid of antigenic property—whether that was due to the previous treatment of the material with formalin or to the poverty of the material itself could not be determined, but the cysts and their contents appeared normal.

Strength of Antigens.

On being tested the extracts of cyst membrane were found to be feeble antigens and were discarded in favour of the alcoholic extracts of scolices. The latter, made in exactly the same way from different batches of material, varied in strength, this apparently depending on the "quality" of the scolices.

At first 10 per cent. alcoholic extracts of moist scolices were employed, but it was found necessary to use them in such low dilutions that there was a distinct danger that the amount of alcohol actually present might

be a factor influencing the result, so that 20 per cent. and finally 25 per cent. extracts were afterwards prepared and used, these allowing of much greater dilution with saline, thus reducing the concentration of alcohol in each tube and avoiding this danger.

With regard to the extracts of dried scolices, those prepared with absolute alcohol gave the most powerful antigens, the antigenic factor being less strong as the alcohol was more diluted with saline for the extraction; this progessive weakening was distinctly noticed when using the 90 per cent., 70 per cent. and 50 per cent. extracts.

Treatment of the scolices with acetone prior to the extraction with alcohol did not reduce markedly the strength of the extract as compared with a similar extract made without this previous treatment; from this it would seem that the antigenic faculty is not soluble to any considerable extent in acetone.

The Standardisation of Extracts.

Each extract was carefully titrated before use to estimate the exact amount which just fixed 3 M.H.D. of complement, the M.H.D. being previously ascertained. The volumes of the various dilutions of antigen (1 : 5, 1 : 7·5, 1 : 10—up to 1 : 25) and of the 3 per cent. suspension of sensitised corpuscles used in the estimation of titre were the same as those employed in the tests according to the method followed (see methods later); in that most frequently used ·5 c.c. was the unit volume of each dilution of antigen pipetted into a series of tubes in ascending dilutions, 3 M.H.D. of complement being then added to each tube, the tubes shaken and incubated for 1½ hours at 37° C., being shaken at half time; ·5 c.c. of the 3 per cent. suspension of sensitised corpuscles was then added to each tube, the tubes shaken and incubated for a further hour, being shaken every 15 minutes. The highest dilution showing no trace of hæmolysis was taken as the titre of the extract under examination and usually worked out at about 1 : 7·5—1 : 10.

The anti-complementary and hæmolytic properties of the extracts.

The low titre usually found—as just stated—showed that the extracts in themselves were not markedly anti-complementary. To test their hæmolytic action ·5 c.c. of the dilution ascertained in standardising the extracts was placed in a tube with ·5 cc. of the 3 per cent. suspension of sensitised corpuscles and incubated for 1½ hours; no hæmolysis of the corpuscles took place. The following Table I. gives the results of titration of three average batches of extracts.

Table II. gives examples of the testing for hæmolytic action.

TABLE I.—TITRATION OF ANTIGENS.

Dilutions ...	1 : 5	1 : 7·5	1 : 10	1 : 12·5	1 : 15	1 : 17·5	1 : 20	1 : 22·5
Batch A.								
Antigen I. ...	o	tr	d	m	vm	c	c	c
,, II. ...	o	o	o	tr	d	m	c	c
,, III....	o	o	ftr	d	m	c	c	c
Batch B.								
Antigen I. ...	o	m	ac	c	c	c	c	c
,, II. ...	o	o	ftr	d	m	c	c	c
,, III....	o	o	tr	m	c	c	c	c
Batch C.								
Antigen I. ...	o	tr	d	vm	ac	c	c	c
,, II. ...	o	o	o	tr	d	vm	c	c
,, III....	o	o	o	tr	m	vm	c	c
90% ...	o	tr	d	vm	ac	c	c	c
70% ...	tr	d	vm	jc	c	c	c	c
50% ...	d	m	ac	c	c	c	c	c

Antigen I. =absolute alcohol extract of moist scolices.
,, II. = ,, ,, ,, dried scolices.
,, III.= ,, ,, ,, acetone-treated scolices.
90%, 70%, 50%=90%, 70%, 50% alcohol extractions of dried scolices.
o=no hæmolysis. ftr=faint trace of hæmolysis. tr=trace of hæmolysis.
d=distinct hæmolysis. m=marked hæmolysis. vm=very marked hæmolysis.
ac=almost complete hæmolysis. jc=just complete hæmolysis. c=complete hæmolysis.

TABLE II.—DETERMINATION OF HÆMOLYTIC ACTION OF ANTIGENS.

Dilutions	1 : 5	1 : 7·5	1 : 10
Batch A.			
Antigen I.	o		
,, II. ...			o
,, III. ...		o	
Batch B.			
Antigen I. ...	o		
,, II.		o	
,, III. ...		o	
Batch C.			
Antigen I. ...	o		
,, II.			o
,, III ...			o

On testing the action of the extracts with syphilitic sera it was found that complete hæmolysis always took place at dilutions of 1 : 20-1 : 24 with 2 and 4 M.H.D. of complement so that by using somewhat higher dilutions—1 : 30 or 1 : 40—the chances of a pseudo-positive reaction with syphilitic sera were avoided ; with 10 per cent. alcoholic extracts the lower dilutions necessary for the test proper might give these pseudo-positive reactions, hence the second advantage of the more concentrated extracts—the first being the avoidance of any possible interference in the results by the concentration of alcohol present.

The emulsification of the extracts.

Both rapid and slow mixing of the alcoholic extracts with the volume of saline necessary to make the total amount of emulsion required at one time were tried in the same series of tests and it was found that a gradual admixture, by gently floating the extract on the surface of the saline, allowing to stand for a while until the layer of junction of the two fluids became milky, slowly rotating the tube until the whole of the alcoholic layer was cloudy, then by more rapid motion completely mixing the two layers, gave a distinctly more turbid emulsion than was obtained by rapidly mixing the two together. These turbid (slowly mixed) emulsions gave slightly but distinctly higher titres than the less cloudy (rapidly mixed) dilutions. Table III.

TABLE III.—SLOW AND RAPID EMULSIFICATION OF ANTIGENS.

Dilutions ...	1 : 5	1 : 6	1 : 7	1 : 8	1 : 9	1 : 10	1 : 12·5	1 : 15
Antigen II. slow	o	o	o	o	ftr	tr	d	m
Antigen II. rapid	o	ftr	tr	tr	d	d	m	ac
Antigen III. slow	o	o	o	ftr	tr	d	m	c
Antigen III. rapid	o	o	o	tr	d	m	ac	c

When set up with horse sera the reaction with the slowly mixed emulsion was distinctly more marked ; on testing the same point with the positive human sera this slight though distinct difference was again noted. Thus a slow admixture of extract and of diluting saline may have some influence on the result of the reaction in the case of sera with only a weak development of antibody.

The effect of the addition of cholesterol.

The next point to be investigated was the usefulness or otherwise of the addition of cholesterol. One per cent. of cholesterol was dissolved in absolute alcohol and added to the extracts in the proportions used in

TABLE IV.—TITRATION WITHOUT AND WITH CHOLESTEROL.

Dilutions ...	1 : 5	1 : 7·5	1 : 10	1 : 12·5	1 : 15	1 : 17·5	1 : 20	1 : 22·5
				Batch D.				
				– *Cholesterol.*				
Antigen I. ...	o	m	ac	c	c	c	c	c
,, II. ...	o	o	ftr	m	ac	c	c	c
,, III....	o	ftr	tr	vm	c	c	c	c
				+ *Cholesterol (1·5 Ext. – 1 Cholesterol).*				
Antigen I. ...	o	tr	m	ac	c	c	c	c
,, II. ...	o	o	o	ftr	m	jc	c	c
,, III....	o	o	o	tr	vm	c	c	c
				+ *Cholesterol (9 Ext. – 1 Cholesterol).*				
Antigen I. ...	o	m	ac	c	c	c	c	c
,, II. ...	o	o	ftr	m	ac	c	c	c
,, III....	o	o	tr	vm	c	c	c	c
				Batch E.				
				– *Cholesterol.*				
Antigen I. ...	ftr	m	c	c	c	c	c	c
,, II. ...	o	ftr	tr	vm	jc	c	c	c
,, III....	o	ftr	m	vm	c	c	c	c
				+ *Cholesterol (1·5 Ext. – 1 Cholesterol).*				
Antigen I. ...	o	tr	vm	c	c	c	c	c
,, II. ...	o	o	ftr	m	jc	c	c	c
,, III....	o	o	ftr	vm	c	c	c	c

the case of the Wassermann heart-cholesterol antigen—viz., three of extract to two of cholesterol—before emulsifying with the saline and the effect on the titres ascertained, two parallel series of tubes being set up ; in the first row the extract alone was used in increasing dilutions, in the second the cholesterol solution had been added to the extract ; a slight but distinct rise of titre was noted as Table IV. shows. In a second series of tests the proportions of extract to cholesterol solution were not 3 : 2 but reduced to 9 : 1 ; this slight proportion of cholesterol solution seemed to have no enhancing effect—two parallel series of tubes being again used *see* Table IV.

A further series was then tried with the proportions of extract to cholesterol starting from 2 : 1, 3 : 1 up to 9 : 1. From 2 : 1 up to 6 : 1 the addition of the cholesterol raised slightly the titre in the standardisation of both the slowly and rapidly emulsified extracts; above the proportion of 6 : 1, up to 9 : 1, the effect of the addition of cholesterol disappeared; this is shown in Table V.

TABLE V.—TITRATION WITH CHOLESTEROL IN VARYING PROPORTIONS.

Dilutions—	1 : 5	1 : 7·5	1 : 10	1 : 12·5	1 : 15	1 : 17·5	1 : 20	1 : 22·5
Antigen II. Batch E.								
(Ext. 2–Ch. 1)	o	o	ftr	m	jc	c	c	c
(Ext. 3–Ch. 1)	o	o	ftr	m	jc	c	c	c
(Ext. 4–Ch. 1)	o	o	ftr	m	jc	c	c	c
(Ext. 5–Ch. 1)	o	o	ftr	m	jc	c	c	c
(Ext. 6–Ch. 1)	o	o	ftr	vm	jc	c	c	c
(Ext. 7–Ch. 1)	o	ftr	tr	vm	jc	c	c	c
(Ext. 8–Ch. 1)	o	ftr	tr	vm	jc	c	c	c
(Ext. 9–Ch. 1)	o	ftr	tr	vm	jc	c	c	c
(–Chol.) ...	o	ftr	tr	vm	jc	c	c	c
Antigen III. Batch D.								
(Ext. 2–Ch. 1)	o	o	o	tr	vm	c	c	c
(Ext. 3–Ch. 1)	o	o	o	tr	vm	c	c	c
(Ext. 4–Ch. 1)	o	o	o	tr	vm	c	c	c
(Ext. 5–Ch. 1)	o	o	o	tr	vm	c	c	c
(Ext. 6–Ch. 1)	o	o	o	tr	vm	c	c	c
(Ext. 7–Ch. 1)	o	o	tr	vm	c	c	c	c
(Ext. 8–Ch. 1)	o	o	tr	vm	c	c	c	c
(Ext. 9–Ch. 1)	o	o	tr	vm	c	c	c	c
(— Chol.) ...	o	o	tr	vm	c	c	c	c

When this point was tested against horse sera the same results were found, viz., that the addition of cholesterol solution in the proportions of 2 : 1 up to 6 : 1 (extract-cholesterol) gave a slightly but definitely greater fixation of complement, whereas above these proportions, *i.e.*, 7 : 1–9 : 1 the enhancing effect of the cholesterol appeared to be lost.

With the human sera from cases of hydatid infection the same held good; both of these were very strongly positive, fixing 3 M.H.D. of complement in dilutions of 1 : 200 and 1 : 600 respectively. From the above results the addition of cholesterol may be of distinct value when testing weakly reacting sera.

Preparation of Materials.

Sera.

Human sera were heated for half-an-hour at 55°C. Rabbit and horse sera were treated in the same way, but with several horse sera the serum controls showed a certain amount of inhibition in addition to the tubes of the test proper; further heating was tried—up to one hour at 55°C. without getting rid of this anti-complementary tendency. The temperature of the inactivation bath was then gradually raised until, after heating for half-an-hour at 60°C. in these particular cases this tendency was destroyed and complete hæmolysis took place.

Unheated sera were set up in some cases and proved strongly anti-complementary.

Complement.

The blood of healthy male guinea pigs was used, the serum was allowed to stand on the clot for four hours, then separated off and stored in the ice chest overnight.

Hæmolytic system.

A 60 per cent. suspension of corpuscles (thoroughly washed) in saline was added to an equal volume of saline containing 10 M.H.D. of hæmolytic serum, these were well and quickly mixed, thus forming a 3 per cent. suspension of sensitised r.b.c. This was allowed to stand at room-temperature for 10 minutes before use. The amount added to each tube depended on the method used for the test (*see* Methods later).

Saline.

·85 per cent. sterile distilled water saline was freshly made for each batch of tests.

Antigen emulsion.

The quantity required was calculated and prepared by slowly mixing the extract with the requisite amount of saline about 10 minutes before use.

Methods employed in the tests and in the preliminary titration of reagents.

Three methods were used in the course of these tests:

1. No. 4 Method as detailed in the Medical Research Committee's Report No. 14, 1918;

2. The Method given in Browning & Mackenzie's "Recent Methods in the Diagnosis and Treatment of Syphilis"; and

3. Fairley's Method as given in his article in "The Quarterly Journal of Medicine,' April, 1922, Vol. XV., p. 244.

In the greater part of the work with alcoholic extracts as antigens the second method was the one adopted, but for economy of material, as the scolex extracts could be made only in small quantities, ·25 c.c. instead of ·5 c.c. was taken as the unit volume for each reagent employed.

The complement was first titrated as follows :—·1 c.c. of guinea pig serum was diluted with ·3 c.c. of saline and ·01, ·02, ·03, ·04 c.c. (= ·0025, ·005, ·0075, ·01 c.c. of pure serum) pipetted into a row of four tubes to each of which ·5 c.c. of the 3 per cent. suspension of red corpuscles was added. The tubes were shaken and incubated for one hour at 37° C., shaking every 15 minutes. Complete hæmolysis occurred generally in the second or third tube.

The amount of antigen emulsion necessary for the batch of tests to be done was prepared, allowing ·25 c.c. per tube. ·25 c.c. of saline was pipetted into each of the "control" tubes (serum, complement and hæmolytic system, but not that for antigen) to take the place of the antigen, ·025 c.c. of the serum to be tested, previously inactivated, was placed in each tube of the test proper and also in the one for serum control; ·25 c.c. of antigen emulsion was next added to each tube containing serum (except the serum control) and also to the one for antigen control; finally the complement in the necessary amount (2, 4, 6, etc., M.H.D. as required) was added to all tubes except the control for the hæmolytic system which contained only ·25 c.c. of saline. The tubes were then gently shaken and incubated at 37°C. for 1½ hours with a shaking at half time. At the end of the 1½ hours ·25 c.c. of the 3 per cent. suspension of sensitised corpuscles was added to all tubes which were shaken and returned to the incubator for a further period of one hour, being again shaken every 15 minutes. At the end of the hour the results were read and again next morning after standing in a cool place all night.

When hydatid fluids were the antigens employed the third method was adopted.

A series of dilutions of complement were prepared, viz., 1 in 2, 4, 6, 8, – – – to 1 in 16 with saline; ·1 c.c. of each of these was pipetted into a series of tubes, ·5 c.c. of saline and ·5 c.c. of sensitised r.b.c. added (the latter prepared by adding 4 M.H.D. of hæmolytic serum to a 3 per cent. suspension of washed r.b.c.) and incubated for half-an-hour at 37°C.

Five tubes were taken for each serum to be tested. ·1 c.c. of pure hydatid fluid was added to each of the first three and ·1 c.c. of serum diluted 1 in 5 was pipetted into the first four; the complement was diluted with saline so that ·1 c.c. contained 3 M.H.D. and ·1, ·15, ·2, ·1, ·1 c.c. added respectively to each tube (*i.e.* 3, 4½ and 6 M.H.D. in the first three—the test proper—and 3 M.H.D. in the fourth and fifth—the serum and complement controls); saline was then added so that the total volume in each tube was ·4 c.c. The tubes were shaken and after incubating for one hour at 37°C. ·1 c.c. of the 3 per cent. suspension of sensitised corpuscles was added to all the tubes which were shaken and incubated for a further half-hour when the readings were taken.

In a number of cases a second series of tubes was set up in which the hydatid fluid content was increased to ·15 c.c. as suggested by Fairley, but this was not found to make any difference to the reaction.

Controls.

In all cases, whichever method was adopted, full controls of the reagents were set up at the same time as the test proper; the serum control tube containing serum, complement and saline but no antigen tested if the serum were properly inactivated, the antigen control tube containing no serum tested if the antigen fixed the complement *per se*, the complement control tube containing neither serum nor antigen served to show that the amount of complement used in the test by itself produced hæmolysis, and the hæmolytic system control tube, containing only saline and the standard volume of sensitised r.b.c. proved that by themselves the r.b.c. showed no trace of hæmolysis.

In addition to the reagent controls one or more negative (normal) sera and a syphilitic serum were set up in the same way as the serum to be tested in order to show whether the antigens used had any binding effect on the complement in the presence of such sera. Finally all sera were also set up against Wassermann antigen (heart-cholesterol).

Application of the Above Methods to Different Sera.

Owing to the difficulty of obtaining sera from human cases of hydatid infection a considerable amount of work was done with animal bloods.

Rabbits.

Six rabbits were inoculated with scolices and brood capsules, several hundreds in one or two inoculations; four were inoculated intraperi-

toneally and two intravenously, the material being obtained from sheep and horse cysts.

After some months, to try and ascertain without sacrificing the animals in the early stages if infection had taken place their bloods were tested with alcoholic extracts—all showed complement fixation. One or two stock rabbits were then tested in the same way with the same results; altogether the sera of 15 uninoculated rabbits were tested and all showed some degree of fixation. The technique was varied slightly; the serum was used in varying volumes from ·05 c.c. down to ·001 c.c.; some specimens showed traces of inhibition as far as ·005 c.c., others only as far as ·02 c.c. Next a fixed amount of serum with varying amounts of complement was used; the majority of sera fixed up to 5 M.H.D. of complement but some inhibited more than others.

A syphilitic and a non-syphilitic (normal) human serum were used as controls and these showed hæmolysis throughout. The rabbit sera were also tested against Wassermann Antigen (heart-cholesterol) and showed no complement-fixation.

These rabbits were all examined at intervals later and no signs of cysts were found. It seems as if rabbit sera may have a tendency to a natural antitody for the "antigen" extracted from hydatid material and if so, this may partly account for the failure in these cases to infect successfully. This work with rabbits proved so anomalous that it was decided to try working with the sera of horses infected with hydatids as far as they could be obtained.

Horses.

Batches of horse bloods were got from the slaughter-houses at intervals, altogether nearly 300 have been examined. These were taken at random at the moment of slaughtering the animals and the carcases examined later for the presence or absence of cysts. Of the first 100 so obtained 10 were from horses with fairly good cysts and 13 from animals showing degenerated ones; the figures were not so high with those collected later, 6 per cent. and 8 per cent. respectively, still there is evidence of a considerable amount of infection among horses so examined. As the positive sera were obtained and tested some were found to give completely negative results (this is referred to later); those which showed some fixation were set up with all the six forms of antigen in varying

dilutions and here the antigens prepared by Methods II. and III. (hereafter called Antigens II. and III.) distinctly showed their superior power of fixation over all those containing any saline; the sera were tested against Wassermann Antigen but in no case showed fixation, and a human negative and a syphilitic serum were set up throughout; these did not show any inhibition with any of the antigens as used. Table VI. gives two examples of this.

TABLE VI.—HORSES.

With Increasing Dilutions of Antigen.

Comp. doses= 3 M.H.D.	*Positive serum II.*			*Positive serum IV.*			
Dilutions—	1 : 30	1 : 40	1 : 50	1 : 30	1 : 40	1 : 50	1 : 60
Antigen I. ...	o	ftr	d	o	o	ftr	m
,, II. ...	o	o	o	o	o	o	tr
,, III....	o	o	tr	o	o	o	tr
,, 90%	o	ftr	d	o	o	tr	m
,, 70%	o	m	ac	o	d	m	ac
,, 50%	d	vm	c	ftr	m	ac	c
Wassermann Antigen ...	c	—	—	c	—	—	—

Controls.	Human		Antigen controls.	Serum controls.	Comp. control.	Hæm system control.
Dilutions 1 : 30	Syphilitic serum.	Negative serum.				
Antigen I. ...	c	c	c	II. c	c	o
,, II. ...	c	c	c	IV. c		
,, III. ...	c	c	c			
,, 90% ...	c	c	c			
,, 70% ...	c	c	c			
,, 50% ...	c	c	c			
Wassermann Antigen ...	o	c	c			

Next, the same antigens were used in a fixed dilution—1 : 30—and the number of M.H.D. of complement varied from 2, 4 – – – up to 10 M.H.D. Here again the superior power of Antigens II. and III. was demonstrated; in one example Antigen II. fixed 6, Antigen III. 4 and Antigen I. 2 M.H.D.; in the second example II. fixed 8, III. 6 and I. 4 M.H.D. In this series of tests also a human normal and a syphilitic serum were set up as controls, in neither case did any fixation occur; the horse sera also gave negative results with the Wassermann Antigen.

Table VII. illustrates this.

TABLE VII.—HORSES.

VARYING DOSES OF COMPLEMENT.

Antigens 1 : 30	*Positive Serum II.*					*Positive Serum IV.*				
Comp. doses ...	2	4	6	8	10	2	4	6	8	10
Antigen I. ...	o	ftr	m	ac	c	o	o	tr	vm	ac
„ II. ...	o	o	o	tr	vm	o	o	o	o	tr
„ III. ...	o	o	ftr	m	c	o	o	o	d	m
„ 90% ...	o	ftr	m	ac	c	o	o	tr	vm	ac
„ 70% ...	o	vm	ac	c	c	o	tr	m	ac	c
„ 50% ...	d	ac	c	c	c	ftr	m	vm	c	c
Wassermann antigen	jc	c	c	c	c	ac	c	c	c	c

Controls.	Syphilitic serum.			Negative serum.			Antigen control.	Serum control.	Comp. control.	Hæm. system control.
Comp. doses ...	2	4	6	2	4	6	2	2	2	2
Antigen I. ...	c	c	c	c	c	c	c	II. c	c	o
„ II. ...	c	c	c	c	c	c	c	IV. c		
„ III. ...	c	c	c	c	c	c	c			
„ 90% ...	c	c	c	c	c	c	c			
„ 70% ...	c	c	c	c	c	c	c			
„ 50% ...	c	c	c	c	c	c	c			
Wassermann antigen	o	o	o	c	c	c	c			

TABLE VIII.—HORSES.

ANTIGENS SLOWLY AND RAPIDLY EMULSIFIED.

Comp doses—3 M.H.D. Antigen II.	Slowly emulsified.						Rapidly emulsified.				
Dilutions—	1 : 30	1 : 40	1 : 50	1 : 60	1 : 70	Serum controls	1 : 30	1 : 40	1 : 50	1 : 60	1 : 70
Positive serum I. ...	o	o	d	m	ac	c	o	tr	m	vm	c
„ „ II. ...	o	o	o	tr	m	c	o	o	tr	m	ac
„ „ III. ...	d	m	ac	c	c	c	m	jc	c	c	c
„ „ IV. ...	o	tr	d	vm	c	c	tr	m	c	c	c
„ „ V. ...	c	c	c	c	c	c	c	c	c	c	c
+ *Cholesterol (Ext. 3–Chol. 2).*											
Positive serum I. ...	o	o	ftr	d	vm		o	ftr	d	m	ac
„ „ II. ...	o	o	o	o	tr		o	o	ftr	m	vm
„ „ III. ...	ftr	ftr	vm	ac	c		d	vm	ac	c	c
„ „ IV. ...	o	o	tr	d	vm		tr	d	vm	c	c
„ „ V. ...	c	c	c	c	c		c	c	c	c	c

Antigen control—c Comp. control—c Hæmolytic system control—o

The effect of slow and rapid emulsification of the extracts was then investigated using varying dilution of the antigens ; here the slow mixing proved of distinct advantage as is shown in Table VIII. ; in the case of sera from infected horses which failed to give a positive reaction the method of mixing made no difference. This was carried a stage further by adding cholesterol to the extract in the proportions used for the

Wassermann Antigen (heart-cholesterol), viz. three parts of extract to two of cholesterol ; here a further accentuation of the reaction was noted, fixation occurring in a higher dilution than when there was no cholesterol present.

TABLE IX.—HORSES.

ANTIGENS WITHOUT AND WITH CHOLESTEROL IN VARYING PROPORTIONS.

Antigen II. 1 : 30. Comp.doses = 3 M.H.D. Proportions of extract—Cholesterol—	1·5 : 1	2 : 1	3 : 1	4 : 1	5 : 1	6 : 1	7 : 1	8 : 1	9 : 1	—Chol.	Serum controls
Horse + ...	o	ftr	ftr	ftr	ftr	tr	m	m	m	m	c
„ + ...	m	m	m	m	m	m	vm	vm	vm	vm	c
„ + ...	tr	tr	tr	tr	tr	tr	d	d	d	d	c
„ + ...	o	o	o	o	o	ftr	tr	tr	tr	tr	c
„ + ...	ftr	ftr	ftr	ftr	ftr	ftr	tr	tr	tr	tr	c
„ + ...	tr	tr	tr	tr	tr	tr	m	m	m	m	c
„ + ...	o	o	o	o	tr	tr	tr	tr	tr	tr	c
„ + ...	o	o	o	o	o	o	o	o	o	o	c
„ + ...	vm	vm	vm	vm	vm	ac	ac	ac	ac	ac	c
„ + ...	o	o	o	o	o	o	o	o	o	o	c
„ + ...	o	o	o	o	o	o	o	o	o	o	c
„ + ...	c	c	c	c	c	c	c	c	c	c	c
„ + ...	c	c	c	c	c	c	c	c	c	c	c
„ + ...	c	c	c	c	c	c	c	c	c	c	c
„ + ...	c	c	c	c	c	c	c	c	c	c	c
„ + ...	c	c	c	c	c	c	c	c	c	c	c
„ + ...	c	c	c	c	c	c	c	c	c	c	c
„ + ...	c	c	c	c	c	c	c	c	c	c	c
„ + ...	c	c	c	c	c	c	c	c	c	c	c
„ + ...	c	c	c	c	c	c	c	c	c	c	c
„ —	c	c	c	c	c	c	c	c	c	c	c
„ —	c	c	c	c	c	c	c	c	c	c	c
„ —	c	c	c	c	c	c	c	c	c	c	c
„ —	c	c	c	c	c	c	c	c	c	c	c
„ —	c	c	c	c	c	c	c	c	c	c	c
„ —	c	c	c	c	c	c	c	c	c	c	c
„ —	c	c	c	c	c	c	c	c	c	c	c
Antigen controls ...	c	c	c	c	c	c	c	c	c	c	c

To further test the effect of the addition of cholesterol the proportions of extract—cholesterol were varied from 1·5 : 1, 2 : 1 – – – – up to 9 : 1, at the same time a tube with no cholesterol being set up ; the antigens were used in a dilution of 1 : 40. Here the effect of cholesterol was distinctly noted up to a proportion of 6 : 1 (in a few cases it ceased at 5 : 1) and from these points up to a dilution of 9 : 1 no enhancing of the reaction was noted. In cases of infected sera showing no complement-fixation with extract alone no addition of cholesterol had any "helping" action as regards fixation.

Table IX. illustrates these points.

Where the sera of infected horses, whether they gave positive or negative reactions with extracts, were set up with hydatid fluids uniformly disappointing results were obtained, no complement-fixation occurring

even when fluids from their own cysts were used. A few examples are given in Table X.

TABLE X.—HORSES.

Sera (giving positive reactions)—tested with hydatid fluids.

				Hyd. fluid control.	Serum control.	Comp. control.	Hæm. system control.
Comp. doses—	3	4½	6	3	3	3	3
Horse II.							
Own fluid	—	—	—	—	—	—	—
Another fluid ...	—	—	—	—			
Horse III.							
Own fluid	—	—	—	—	—	—	—
Another fluid ...	—	—	—	—			
Horse V.							
Own fluid	—	—	—	—	—	—	—
Another fluid ...	—	—	—	—			

Infected horses (giving negative reactions)—tested against hydatid fluids.

Sera.							
1 Own fluid ...	—	—	—	—	—	—	—
2 ,, ,, ...	—	—	—	—	—	—	—
3 ,, ,, ...	—	—	—	—	—	—	—
3 Fluid A ...	—	—	—	—			
3 ,, B ...	—	—	—	—			
4 ,, C ...	—	—	—	—	—	—	—
4 ,, D ...	—	—	—	—			
4 ,, E ...	—	—	—	—			
5 ,, F ...	—	—	—	—	—	—	—
5 ,, G ...	—	—	—	—			

The sera of six horses reported uninfected gave anomalous results, almost complete or partial fixation occurring not only in the tubes with antigen but also in the serum controls, and these were tested further. The sera were heated for longer periods,from 40 minutes to one hour at 55°c. and each batch re-tested but the same partial fixation was still noticed. The temperature of inactivation was then gradually raised and the sera re-tested until, after heating half-an-hour at 60°c., this faculty of complement-fixation was lost and the reaction showed complete hæmolysis throughout, both in the tubes of the test proper and in the serum controls. Whether a possible explanation of this phenomenon in the case of certain horses may be that there is present in their serum some strongly anti-

complementary property which is not destroyed at 55°c. but is got rid of at 60°c. is an interesting point, but it seems to render the test at times unreliable as a means of diagnosis for these animals if such a test should be considered advisable. Table XI. gives examples of these sera—after heating at 55°c., and also at 60°c. for half-an-hour.

TABLE XI.—HORSES.

Uninfected sera giving anomalous reactions.

Comp. doses=3 M.H.D.	Heated at 55° C.		Heated at 60° C.	
Antigen 1 : 30		Serum control.		Serum control.
Serum A	ftr	ftr	c	c
„ B	d	d	c	c
„ C	m	m	c	c
„ D	vm	vm	c	c
„ E	ftr	ftr	c	c
„ F	o	o	c	c

Summary of the work with horses.

The sera of infected horses failed to give a positive reaction with any hydatid fluids that could be procured whether from their own cysts or not.

With alcoholic extracts as antigens somewhat varied results were obtained ; some of the infected horses gave quite good positive reactions, in others the reaction was slight while in still other examples it failed. In the case of those showing no fixation possible explanations may be—(1) the large bulk of the host in comparison with the size and number of the parasites, the development of antibody not being great enough to permeate the blood stream sufficiently to give a " naked eye " demonstration, or (2) the development of efficient impermeable barriers by the host round the cysts. In this connection it may be mentioned that in no case was a very heavy infection reported for a positive blood sent in, the majority having from 2–4 or 5 medium sized cysts. Three horses among those slaughtered were afterwards found to be harbouring several very large cysts varying from the size of an orange up to that of a child's head, but unfortunately in these cases the blood had not previously been collected as it so happened, the collection being done at random before slaughtering.

Six negative sera gave more or less strongly positive reactions which disappeared when the sera were heated to 60°C. The slow mixing of the antigen emulsion and the addition of cholesterol proved of help in intensifying the reaction with positive sera.

Cattle sera.

A few samples of blood were obtained but unfortunately in no case was infection with hydatids discovered on examination afterwards so that a positive serum could not be tested; the negative sera were, however, set up with the various extracts and showed no trace of fixation.

Human sera.

Eight bloods were sent in at different times for diagnosis during the course of this work; seven of these showed no complement-fixation either with hydatid fluids or alcoholic extracts as antigens, two or more of both of these being employed in each case. The further history of six of these cases is unknown; in the seventh case an operation was performed shortly afterwards revealing "an alveolar sarcoma" apparently of a primary lung origin; the patient died and the postmortem revealed nothing further.

In the eighth case, where a cyst had been removed some years previously and a further infection was suspected, strongly positive reactions were obtained with alcoholic extracts and a weak reaction with two hydatid fluids out of three.

A specimen from another infected case was obtained through the kind offices of Dr. Bannerman, County Medical Officer of Health for Orkney, who went to considerable trouble to obtain it, to whom I tender my best thanks. This case also gave strongly positive reactions. In these two positive cases all six forms of extract were used as well as hydatid fluids. Details of these two cases follow.

Case A. Aged 50. Operated on twice in 1910 with an interval of three weeks between; the patient reported "innumerable cysts were found—irremovable." When the blood was sent for the present test the patient was confined to bed in poor condition, complaining of abdominal pain and cramp in the legs.

In this case the serum was first of all tested against all six antigens in varying dilutions from 1 : 60, increasing up to 1 : 225. The point which stands out here is the much greater efficacy of Antigens II. and III.

over the others, which all contained some saline in the alcohol used for extraction. Antigen II. proved a little more powerful than III., giving complete fixation up to 1 : 200, whereas III. showed traces of hæmolysis at that point. Case A.—Table I. shows this.

CASE A.—TABLE I.

INCREASING DILUTIONS OF ANTIGEN.

Comp. doses = 3 M.H.D. Dilutions—	1 : 60	1 : 70	1 : 80	1 : 90	1 : 100	1 : 125	1 : 150	1 : 175	1 : 200	1 : 225
Antigen I. ...	o	o	o	o	o	m	c	c	c	c
„ II. ...	o	o	o	o	o	o	o	o	o	d
„ III. ...	o	o	o	o	o	o	o	o	tr	m
„ 90% ...	o	o	o	o	o	m	c	c	c	c
„ 70% ...	o	o	o	o	tr	vm	c	c	c	c
„ 50% ...	o	o	tr	d	vm	c	c	c	c	c

Controls.

Antigen 1 : 40	Syphilitic serum.		Negative serum.		Antigen controls.		Comp. control.	Hæm. sys. control.	Serum control.
Comp. doses—	2	4	2	4	2	4	2		2
Antigen I.	c	c	c	c	c	c	c	o	c
„ II.	c	c	c	c	c	c			
„ III.	c	c	c	c	c	c			
„ 90%	c	c	c	c	c	c			
„ 70%	c	c	c	c	c	c			
„ 50%	c	c	c	c	c	c			
Sera controls	c		c						

The next stage was to take a fixed dilution of the antigen, 1 : 60, and test it out against increasing M.H.D.s of complement, starting from 3 up to 24 M.H.D. With Antigen II. 15 M.H.D. were fixed and with Antigen III. 12, whereas with the other four antigens fixation was markedly less, decreasing as the amount of saline mixed with the alcohol increased. A further point now tried was to take Antigens I., II. and III.

CASE A.—TABLE II.

Increasing doses of complement.

Antigen 1 : 60									Antigen controls	
Comp. doses—	3	6	9	12	15	18	21	24	2	4
Antigen I.	o	o	o	m	c	c	c	c	c	c
„ II.	o	o	o	o	o	m	vm	c	c	c
„ III.	o	o	o	o	ftr	vm	c	c	c	c
„ 90%	o	o	o	m	c	c	c	c	c	c
„ 70%	o	o	d	vm	c	c	c	c	c	c
„ 50%	o	tr	m	c	c	c	c	c	c	c
Comp. control	c									
Hæm. system control ...	o									
Serum control	c									

at the highest dilutions in each case showing complete inhibition with 3 M.H.D. and to use an increasing series of M.H.D., when II. at 1 : 200

was found to fix six, whereas I. and III. just failed to fix six at their maximum dilutions of 1 : 100 and 1 : 175 respectively. These points are shown in Case A—Tables II. and III.

CASE A.—TABLE III.

Antigens at highest inhibiting dilutions, with increasing doses of complement.

Comp. doses	3	6	9	12	15
Antigen I. @ 1 : 100	o	tr	vm	c	c
,, II. @ 1 : 200	o	o	tr	m	c
,, III. @ 1 : 175	o	ftr	m	c	c

CASE A.—TABLE IV.

With Hydatid Fluids.

Comp. doses.		3	4½	6	Ant. control. 3	Sera controls. 3	Comp. control. 3	Hæm. system control.
Fluid 1 ...	Serum A ...	+ + +	+ + —	± — —	— — —	— — —	— — —	+ + +
	Negative serum	— — —	— — —	— — —		— — —		
Fluid 2 ...	Serum A ...	— — —	— — —	— — —	— — —			
	Negative serum	— — —	— — —	— — —				
Fluid 3 ...	Serum A ...	+ + —	+ — —	— — —	— — —			
	Negative serum	— — —	— — —	— — —				

+ + + = no hæmolysis. + — — = marked hæmolysis. — — — = complete hæmolysis.
+ + — = slight ,, ± — — = almost complete hæmolysis.

CASE A.—TABLE V.

With Wassermann Antigen.

Antigen 1 : 30.		Sera controls.	Antigen control.	Comp. control.	Hæm. sys. control.
Comp. dose = 4 M.H.D.					
Serum A	c	c	c	c	o
Negative serum ...	c	c			
Syphilitic serum ...	o	c			

CASE A.—TABLE VI.

Slow and Rapid Mixing, without and with Cholesterol.

	Without Cholesterol.			*With Cholesterol.*		
Antigen II. dilutions ...	1 : 200	1 : 225	1 : 250	1 : 200	1 : 225	1 : 250
Slowly mixed	o	d	m	o	o	tr
Rapidly mixed	tr	m	ac	o	tr	m
Antigen controls ...	c	c	c	c	c	c

The serum was tested with hydatid fluids as antigens, three different fluids being employed; one showed no trace of inhibition, one gave complete fixation with three and partial with 4½ M.H.D., and the third gave partial fixation with 3 M.H.D. only. See Case A—Table IV.

The serum was also set up with Wassermann Antigen (heart-cholesterol) in the dilution of 1 : 30, with negative results. Case A—Table V. shows this.

Finally the effect of the rapid and slow mixing of the emulsion was tested with Antigen II. starting at 1 : 200 ; the slow mixing gave a distinct but slight enhancing of fixation—see Case A—Table VI. Unfortunately this used up the last of the serum and the test could not be carried any further. Later a second specimen was asked for but the man was too ill for it to be taken.

Case B. Operated on in 1912 when a cyst was removed from the liver, a second cyst (suppurating) was removed in 1921. At the time of this test he complained of pain in the right hypochondrium ; x-ray examination revealed (?) bulging diaphragm.

Three series of tests were done in this case, all giving strongly positive results. Shortly afterwards the patient was operated on and another cyst removed.

CASE B.—TABLE I.
INCREASING DILUTIONS OF ANTIGEN.

Comp. dose=3M.H.D. Dilutions	1 : 60	1 : 80	1 : 100	1 : 125	1 : 150	1 : 175	1 : 200	1 : 300	1 : 400	1 : 500	1 : 600	1 : 800
Antigen I.	o	o	o	o	o	o	o	o	tr	m	c	c
,, II.	o	o	o	o	o	o	o	o	o	o	o	tr
,, III. ...	o	o	o	o	o	o	o	o	o	o	o	d
,, 90% ...	o	o	o	o	o	o	o	o	tr	m	c	c
,, 70% ...	o	o	o	o	o	o	tr	m	vm	c	c	c
,, 50% ...	o	o	o	o	o	tr	m	ac	c	c	c	c

Controls.

Antigen 1 : 40	Syphilitic serum.		Negative serum.		Antigen controls.		Comp. control.	Hæm. sys. control.	Serum control.
Comp. doses—	2	4	2	4	2	4	2		2
Antigen I.	c	c	c	c	c	c	c	o	c
,, II.	c	c	c	c	c	c			
,, III.	c	c	c	c	c	c			
,, 90%	c	c	c	c	c	c			
,, 70%	c	c	c	c	c	c			
,, 50%	c	c	c	c	c	c			
Sera controls	c		c						

The same procedure of tests was followed as with Case A ; the serum was first tested against the six antigens in dilutions which had to be continued to 1 : 800 before traces of hæmolysis were shown with Antigens II. and III., rather more distinct in the latter. This test confirmed the results found with Case A as regards the superiority of Antigens II. and III. Case B—Table I. shows this.

On setting up the serum with antigens at a fixed dilution 1 : 60, and using increasing strengths of complement from 3—27 M.H.D., it was found that with Antigen II. 18 M.H.D. were fixed, and with Antigen III. the same amount, but there was a slight difference in the amount of hæmolysis present in the next tube—containing 21 M.H.D. ; here again the extracts containing some admixture of saline showed the same lessening of power of fixation, lessening progressively with the increasing proportion of saline present. See Case B—Table II.

With the highest dilutions of Antigens I., II. and III. showing complete absence of hæmolysis with 3 M.H.D., II. and III. just fixed six and showed traces of hæmolysis with 9 M.H.D., while with I. there was distinct hæmolysis with 6 M.H.D. Case B—Table III. illustrates this point.

CASE B.—TABLE II.

Increasing Doses of Complement.

Antigens 1 : 60.										Antigen controls.	
Comp. doses—	■	6	9	12	15	18	21	24	27	2	4
Antigen I.	■	o	o	o	tr	d	vm	c	c	c	c
,, II.	o	o	o	o	o	o	ftr	d	m	c	c
,, III.	o	o	o	o	o	o	tr	d	vm	c	c
,, 90%	o	o	o	o	d	vm	c	c	c	c	c
,, 70%	■	o	o	tr	m	ac	c	c	c	c	c
,, 50%	o	o	d	vm	c	c	c	c	c	c	c
Comp. control ...	c										
Hæm. sys. control... ...	■										
Serum control	c										

CASE B.—TABLE III.

Antigens at Highest Inhibiting Dilutions, with increasing Doses of Complement.

Comp. doses	3	6	9	12	15	18	21
Antigen I. @ 1 : 300...	o	d	m	c	c	c	c
,, II. @ 1 : 600...	o	o	ftr	d	ac	c	c
,, III. @ 1 : 600...	o	o	tr	m	ac	c	c

CASE B.—TABLE IV.

With Hydatid Fluids.

Comp. doses.		3	4½	6	Antigen control. 3	Sera controls. 3	Comp. control. 3	Hæm. sys. control.
Fluid 1...	Serum B... ...	+ + +	+ + +	+ — —	— — —	— — —	— — —	+ + +
	Negative serum...	— — —	— — —	— — —		— — —		
Fluid 2...	Serum B... ...	+ + —	— — —	— — —	— — —			
	Negative serum...	— — —	— — —	— — —				
Fluid 3...	Serum B... ...	— — —	— — —	— — —	— — —			
	Negative serum...	— — —	— — —	— — —				
Fluid 4...	Serum B... ...	+ + —	— — —	— — —	— — —			
	Negative serum...	— — —	— — —	— — —				

On testing the serum with four different hydatid fluids it was found that one fluid completely fixed 4½ and showed only slight fixation with 6 M.H.D., a second fluid gave completely negative results and the other two showed traces of hæmolysis with 3 M.H.D. See Case B—Table IV.

The last step was to set up the serum with Wassermann Antigen (heart-cholesterol) diluted 1 : 30, with negative results, as shown in Case B—Table V. The usual controls were used throughout and a syphilitic and a negative serum were also set up.

CASE B.—TABLE V.

WITH WASSERMANN ANTIGEN.

Antigen 1 : 30.			Serum control.	Comp. control.	Antigen control.	Hæm. sys. control.
Comp. doses—	2	4	2	2	2	
Serum B...	c	c	c	c	c	o
Syphilitic serum ...	o	o	c			
Negative serum... ...	c	c	c			

CASE B.—TABLE VI.

SLOW AND RAPID MIXING, WITHOUT AND WITH CHOLESTEROL.

Antigen 1 : 60.					Syphilitic serum.		Negative serum.	
Comp. doses—	18	21	24	27	2	4	2	4
Slowly mixed ...	o	tr	d	m	c	c	c	c
Rapidly mixed ...	tr	d	vm	ac	c	c	c	c
With Cholesterol.								
Slowly mixed ...	o	o	tr	d	c	c	c	c
Rapidly mixed ...	ftr	tr	m	ac	c	c	c	c

Antigen control—slow—c. Rapid—c. Comp. control—c. Serum control—c. Hæm. system control—o.

Finally the question of the slow and rapid emulsification of the extract, using Antigen II., was tested with dilutions of antigen from 1 : 600—1 : 800; a distinct retarding of the commencement of hæmolysis was noted with the slowly mixed as compared with the rapidly mixed emulsion. On adding cholesterol and again mixing both slowly and rapidly a further slight increase of fixation was noted as is shown in Case B—Table VI.

The last point that could be taken up with the remains of the serum was to test the same two points—slow and rapid mixing without and with cholesterol—with a fixed dilution of Antigen II., viz. 1 : 60 and increasing M.H.D. of complement; the same slight but sufficiently distinct differences were noted. In this last test a normal and a syphilitic serum were set up, both showing in all cases absolutely complete hæmolysis. Case B.—Table VI. gives this.

Summary of the two cases in man.

In both cases the reactions with the hydatid fluids that could be obtained varied from complete absence of fixation to the fixation of 4½ M.H.D., the highest result shown.

Far more strongly marked reactions were noted with the alcoholic extracts ; the extract giving the poorest results was the one made with 50 per cent. alcohol ; as the strength of the alcohol increased the reaction became stronger whether considered from the point of the highest dilution fixing 3 M.H.D. of complement or from that of the number of M.H.D. fixed by a definite dilution of antigen. With the absolute alcohol extracts of dried scolices these reactions reached their maximum, comparatively speaking, and there was only a slight diminution when the scolices had been treated with acetone before being extracted with alcohol.

The slow and rapid mixing and the addition of cholesterol were also tested, these two points being specially indicated in the work with horse sera. The addition of cholesterol had a distinctly enhancing effect on the reaction and the rapid mixing of the emulsion slightly lessened its potency, whether made from extract alone or with the addition of cholesterol. Neither serum showed any tendency to fixation with Wassermann Antigen nor did the syphilitic sera used as controls show any fixation with any of the antigens in the dilutions employed.

General Summary of Results.

1. Alcoholic extracts of scolices were found to be more potent antigens than the hydatid fluids usually obtainable in this country.

2. Extracts prepared from dried scolices with absolute alcohol were the most potent.

3. The treatment of the scolices with acetone previous to the extraction with alcohol was found to only slighten lessen the antigenic power of the extract.

4. The presence of saline in the alcohol for extraction lessens the potency of the extract.

Considering these three points 2–4 the antigenic factor seems to be alcohol-soluble rather than acetone or saline-soluble, and to be mainly lipoidal in nature.

5. The possible interference of alcohol in the reaction was avoided by making the extracts of a sufficiently high concentration to require considerable dilution with saline before use.

6. These concentrated extracts in the same way lessened the danger of pseudo-positive reactions with syphilitic sera.

7. The slow emulsification of the extract with the diluting saline was of advantage, giving a rather stronger reaction than when the emulsion was rapidly made.

8. The addition of cholesterol within the limits of 1 part of cholesterol to 1½ to 6 parts of extract was also of distinct advantage in enhancing the reaction.

These last two points may prove of value in the diagnosis of weakly reacting sera.

ACKNOWLEDGMENT.

This work has been done, with the help of a grant from the Medical Research Committee, under Professor R. T. Leiper's direction in his laboratory at the London School of Tropical Medicine and Hygiene. To him, and to Dr. H. H. Scott, who has offered much helpful advice and criticism, I tender my best thanks. I had also the opportunity of working for a fortnight in Professor C. H. Browning's laboratory at Glasgow University, and I express my grateful thanks for the facilities afforded there.

REFERENCES.

1906-7.

GHEDINI, G.—*Gaz. degli ospedali e delle cliniche*, 23/12/06,, XXVII., p. 1616.
GHEDINI, G.—*Gaz. degli ospedali e delle cliniche*, 1907, XXVIII. p. 53.'

1908.

APPHATIE, A. and LORENZ, E.—*Semana medica*, Buenos Aires, 15/10/08, XV., p. 167.
WEINBERG, M. and PARVU, M.—*C. R. Soc. Biol.*, 1908, LXV., pp. 298, 561 and 644.

1909.

Apphatie, A. and Lorenz, E.—*Tribune médicale*, 1909, p. 229.
Bettencourt, N.—*Arch. do Inst. bact. Camara Pestana*, 1909, II., 3, p. 36.
Colombani, F.—*Wien klin. Wchnschr*, 1909, XXII., p. 1413.
Durand, L.—*Diag. de l'Echinococcose.—Thesis*, Paris, 1909.
Fleig, C. and Lisbonne, M.—*Presse médicale*, 7/8/09, 63, p. 832.
de Gaetano, L.—*Policlinico, sez. prat.*, 1909, p. 1524.
Jianu, A.—*Wien klin. Wchnschr*, 1909, XXII., 42, p. 1439.
Kreuter, E.—*Münch. med. Wchnschr*, 1909, LVI., 36, p. 1828.
Lejars, F., Delbet, P., Faure, J.L.—*Semaine médicale*, 31/3/09, XXIX., 13, p.152.
Lejars, F. and Parvu, M.—*Bull. et Mém. Soc. de chir*, 24/3/09, XXXV., pp. 403 and 850.
Paisseau, G., and Tixier, L.—*Presse médicale*, 1909, XVII., 80, p. 697.
Parvu, M.—*C. R. Soc. Biol.*, 1909, LXVI., p. 767.
Parvu, M. and Laubry, Ch.—*C. R. Soc. Biol.*, 1909, LXVI., p. 467.
Putzu, I.—*Biochim. e. Terap. Sper.*, 1909, I., p. 385.
Rossello, H.—*Presse médicale*, 7/8/09, 63, p. 561.
Walther, Ch.—*Semaine médicale*, 7/4/09, XXIX., 14, p. 167.
Walther, Ch.—*Bull. et Mém. Soc. de Chir.*, 1909, XXXV., p. 422.
Weinberg, M.—*Ann. de l'Inst. Pasteur*, 25/6/09, XXIII., 6, p. 472.
Weinberg, M.—*C. R. Soc. Biol.*, 1909, LXVI., pp. 133, 539 and 816.
Weinberg, M. and Boidin, L.—*C. R. Soc. Biol.*, 1909, LXVI., 3, p. 135.
Weinberg, M. and Vieillard.—*Bull. Soc. Cent. de Méd. vét.*, 1909, LXIII., p. 50.

1910.

Bettencourt, N.—*C. R. Soc. Biol.*, 18/6/10, LXVIII., p. 1066.
Braunstein, G.—*Wien klin. Wchnschr*, 1910, XXIII., p. 1139.
Brugnatelli, E.—*Pathologica*, 1910, p. 388.
Chauffard, A. and Vincent, Ch.—*Gaz. des hôpitaux*, 1/3/10, p. 343.
Chauffard, A. and Vincent, Ch.—*Bull. et Mém. Soc. méd. des hôpitaux*, 24/6/10, p. 859.
Dobrotine, A. N.—*Berl. klin Wchnschr*, 1910, XLVII., p. 1315.
Eckenstein, K.—*Lancet*, 6/8/10, II., p. 377.
Graetz, F.—*Centralbl. f. Bakt. Orig.*, 1910, LV., 3, p. 234.
Israel, A.—*Ztschr. f. Hyg. u. Infektionskr*, 1910, LXVI., p. 487.
Laubry, Ch. and Parvu.—*Bull. et Mém. Soc. méd. des hôpitaux*, 11/11/10, XXVI., p. 412.
Lippmann, H.—*Berl. klin Wchnschr*, 1910, XLVII, 1, p. 13.
Maraninó, G.—*Rev. clin. de Madrid, Med.-spec.*, 1910, III., p. 201.
Meyer, K.—*Berl. klin. Wchnschr*, 1090, XLVII., 28, p. 1316.
Meyer, K.—*Ztschr. f. Immünitätsforsch*, 1910, VII., 6, p. 732.
Pevnitzsky, A. A.—*Journ. Méd. milit.*, 1910, VI., p. 290.
Pevnitzsky, A. A.—*Voyen-med. J. St. Petersburg, med. spec.*, 1910, CCXXVIII., p. 290.
Puntoni, V.—*Bull. d. Sc. med.*, 1910, X., p. 245.
Puntoni, V.—*Bull. d. Sc. de Bologna*, 1910, VIII., p. 123.
Putzu, J.—*Centralbl. f. Bakt. orig.*, 1910, LIV., 1, p. 77.
Schoo, H. J. M.—*Nederl. Tijd. v Geneesk*, 1910, XIV., 1, p. 1441.

THOMSEN, O. and MAGNUSSEN, G.—*Arch. f. klin. Chir.*, 1910, XCI., p. 100.
WEINBERG, M.—*C. R. Soc. Biol.*, 1910, LXVIII., p. 446.
ZAPELLONI, L. C. and RICCIUTI, G.—*Biochim. e terap. sper.*, 1910, II., 6, p. 241.

1911.

BETTENCOURT, N.—*Arch. do Inst. bact. Camara Pestana*, 1910 and 11, III., p. 219.
BRAUER, A.—*Münch. med. Wchnschr*, 16/5/11, LVIII., 20, p. 1073.
BRAUER, A.—*Deut. med. Wchnschr*, 1911, XXXVII., p. 1440.
BUSSON, B.—*Centralbl. f. Bakt. orig.*, 1911, LX., 5, p. 426.
HENIUS, K.—*Deut. med. Wchnschr*, 1911, XXXVII., 26, p. 1212.
ISRAEL, A.—*Münch. med. Wchnschr*, 1911, LVIII., p. 1567.
KREUTER, E.—*Beit. z. klin. chir. Tübing.*, 1911, LXXVI., p. 829.
KREUTER, E.—*Münch. med. Wchnschr*, 1911, LVIII., 32, p. 1747.
LEGRY, T. and PARVU, M.—*Presse médicale*, 4/11/11, XIX., 88.
MEYER, K.—*Ztschr. f. Immünitätsforsch*, 1911, IX., p. 530.
URIOSTE, J. P. and SCALTRITTI, A.—*Presse médicale*, 25/1/11, 7, p. 57.
VAS, B.—*Wien med. Wchnschr*, 1911, LXI., 4, p. 251.
WEINBERG, M.—*C. R. Soc. Biol.*, 1911, LXIX., p. 229.

1912.

BARSONY, T. and EGAN, E.—*Ztschr. f. klin. Med.*, 1912, LXXVI., p. 269.
FALCOIANO.—*Ann. de Méd.-vét.*, 1912, LXI., p. 617.
GRAETZ, F.—*Ztschr. f. Immünitätsforsch*, 1912, X., 1., p. 60.
HAHN, B.—*Münch. med. Wchnschr*, 1912, LIX., 25, p. 1483.
HERTZ, R.—*Deut. med. Wchnschr*, 1912, XXXVIII., 30, p. 1418.
PARVU, M.—*C. R. Soc. Biol.*, 16/3/12, LXXII., p. 462.
PFEILER, W.—*Ztschr. f. Infektionskr. d. Haustiere*, 1912, II., pp. 70, 153 and 255.
DE SANDRO, D.—*Riforma medica, Napoli*, 23/3/12, XXVII., 12, p. 309.
THOMSEN, O. and MAGNUSSEN, G.—*Berlin klin. Wchnschr*, 1912, XLIX., 25, p. 1183.
WEINBERG, M.—*Ann. de l'Inst. Past.*, 1912, XXVI., p. 424.

1913.

ABRIKOSOW, A.—*Medic. Obozren*, 1913, LXXIX., 7, p. 603.
FALCOIANO.—*Centralbl. f. Bakt.*, 1913, Ref. 1, p. 138.
SILBER, S. and CHMELINSKY, B.—*Charkower Med. J.*, 1913, XVI., p. 22.
SONNTAG, E.—*Beitr. z. klin. Chir*, 1913, LXXXII., p. 454.
WEINBERG, M.—*Kolle u. Wassermann "Handbuch der pathogenen Mikroorganismen,"* 1913, VIII., p. 123.

1914.

GIANI, E.—*Folio clin. chim. et micros.*, 1912-14, IV., p. 320.
SALVAT, A.—*Semana medica*, 1914, XXXII., 2, p. 914.

1915.

ZAPELLONI, L. C.—*Policlinico sez. chir.*, 1915, XXII., p. 321.

1916.

KOLMER, J. A., TRIST, M. E. and HEIST, G. D.—*J. Infect. Dis.*, 1916, XVIII., 1, p. 88.

1919.

DUBOT, E.—*C. R. Soc. Biol.*, 1919, LXXXII., p. 746.

1920.

Apphatie, A. and Lorenz, E.—*Semana medica*, 2/9/20, xxvii., 36, p. 303.
Jahn, E.—*Zeitschr. f. Fleisch u Milchhygiene*, 1/4/20, xxx., 13, p. 173.
Pontano, F.—*Policlinico sez. med.*, 11/20, xxvii., 11, p. 405.

1921.

Blumenthal, G.—*Berl. klin. Wchnschr*, 5, p. 21, lxviii., 36, p. 1067.
Fairley, N. H.—*Med. J. Aust.*, 12/3/21, i., 11, p. 205.
Luridiana, P.—*Policlinico sez. prat.*, 10/21, xxviii., 2, p. 41.
Magath, T. B.—*Med. Clin. N. Am. Phila.*, 1921, v., p. 549.
Pewny, W.—*Wien klin. Wchnschr*, 1921, xxxiv., 33, p. 402.
Rubinstein, M.—*Traité pratique de Sérologie*, Paris, 1921.

1922.

Albo, M.—*Surg., Gyn. and Obstet.*, 1922, xxxiv., p. 739.
Deluca, F.—*C. R. Soc. Biol.*, 1922, lxxxviii., p. 346.
Fairley, N. H.—*Quart. J. Med.*, 4/22, xv., 39, p. 244.
Ithurrat, R. F. and Calcagno, B. N.—*Semana medica*, 1922, xxix., 2, p. 857.
Torto, P.—*Riforma medica, Napoli*, 1922, xxxviii., p. 867.

1923.

Blumenthal, G. and Unger, E.—*Deut. med. Wchnschr*, 20/4/23, xlix., p. 513.
Fairley, K. D.—*Med. J. Aust.*, 14/7/23, ii., 2, p. 27.
Flssneõr, O.—*Ztschr. f. Biol.*, 1923, lxxx., p. 255.
Hoeden, J. van der.—*Deut. med. Wchnschr*, 24/8/23, xlix., 34, p. 1108.
Ithurrat, R. F. and Calcagno, B. N.—*Presse médicale*, 5/9/23, xxxi., p. 71.
Patterson, S. W. and Williams, F. E.—*Med. J. Aust.*, 3/11/23, ii., p. 467.
Rabinowitsch, M.—*Centralbl. f. Bakt. orig.*, 1923, xci., p. 102.
Thiellé, Th.—*Arch. Inst. Pasteur*, Tunis, 1923, xii., 3 and 4, p. 313.

1924.

Bryce, L. M., Kellaway, C. H. and Williams, F. E.—*Aust. J. Exper. Biol.*, 1924, i., p. 77.
Dew, H. R. and Williams, F. E.—*Med. J. Aust.*, 15/3/24, Suppt. i., 113.
Flössner, O. and Kellner, A.—*Münch. med. Wchnschr*, 10/10/24, lxxi., 41, p. 1427.
Goudsmit, J.—*Nederl. Tijdschr. v. Geneesk*, 6/9/24, lxviii., p. 1235.
Hoeden, J. van der.—*Münch. med. Wchnschr*, 18/1/24, lxxi., 3, p. 77.
Mogena, H. G.—*Arch. esp. de Enf. del ap. Digest.*, Madrid, 6/24, vii., p. 345.
Patterson, S. W. and Williams, F. E.—*J. Path. and Bact.*, 9/1/24, xxvii., p. 1.
Trenti, E.—*Policlinico sez. med.*, 1/7/24, xxxi., 7, p. 353.'

1925.

Deusch, G.—*Deut. med. Wchnschr*, 7/8/25, li., p. 1319.
Dew, H. R., Kellaway, C. H. and Williams, F. E.—*Med. J. Aust.*, 9/5/25, i., 19, p. 471.
Flössner, O.—*Ztschr. f. Biol.*, 1925, lxxxii., p. 297.
Jaffé, L.—*Deut. med. Wchnschr*, 10/7/25, li., 28, p. 1156.
Kellaway, C. H.—*Med. J. Aust.*, 25/4/25, i., 17, p. 417.
Sette, N.—*Policlinico sez. prat.*, 24/8/25, xxxii., 34, p. 1175.

1926.

Horowitz-Wlassowa, L.—*Deut. med. Wchnschr*, 22/1/26, lii., p. 147.

Journal of Helminthology, Vol. IV., *Nos.* 4-5, *November*, 1926, *pp.* 179-182.

Helminths collected from Horses in the Aberystwyth Area.

By E. Aneurin Lewis, M.Sc.

(*Demonstrator, University College of Wales, Aberystwyth, and Field Officer, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

In February, 1924, Dr. Walton recorded *Anoplocephala perfoliata, Ascaris equi, Oxyuris equi* (*curvula*), and *Cylicostomum tetracanthum* from horses in North Wales; and in January 1925, Mr. Bissett, M.R.C.V.S., found, in a horse from Duffryn Mountains (South Wales), a large number of parasitic worms, including *Anoplocephala mammillana, Cylicostomum tetracanthum, Strongylus edentatus, S. vulgaris, S. equinus,* and one specimen of *Oxyuris*. These, it seems, are the only recent records concerning helminthic parasites of horses in Wales. It was therefore thought desirable to examine a few horses very carefully.

In February 1925 a large number of worms were collected from a horse which had died at a farm near Tregaron (Cardiganshire); and in March and April 1925 the writer received the kind permission of Sir Lewes Pryse, of Gogerddan, near Aberystwyth, to examine two horses which were slaughtered at the Gogerddan Kennels.

The number of helminths collected from each of these three cases was very large.

In the material there were 24 species of helminthic worms. These included 21 species of Strongyles, one Ascarid, one Oxyurid and one Anoplocephala.

Of the Strongyles 15 are new records for Wales. It may be noted here that Bissett records *Anoplocephala mamillana* from a horse in South Wales. This species has not been found in this district.

A list of the species found is given and remarks are added concerning each species.

CESTODA.

ANOPLOCEPHALA PERFOLIATA (Goeze, 1782) E. Blanchard, 1848.

Large numbers of this species were found in two horses. They were obtained from all regions of the intestines, and from the cæcum. From one horse over 1,000 *A. perfoliata* were counted. More were collected but not counted.

NEMATODA.

ASCARIS EQUORUM Goeze, 1782.

This species was obtained from the intestines of two horses. Two specimens were obtained from one ; and three specimens from the other. In the former case one Ascarid was collected from the cæcum.

OXYURIS EQUI (Schrank, 1758) E. Blanchard, 1849.

This parasite was found in the intestines of one horse, from which only five were obtained.

STRONGYLUS (STRONGYLUS) EQUINUS (Müller, 1780 *ex parte*).

A parasite occurring in very large numbers in the right ventral colon and the cæcum of all three horses.

STRONGYLUS (ALFORTIA) EDENTATUS (Looss, 1900).

Large numbers were obtained from the right ventral colon. It occurred, less abundantly, also in other parts of the intestine.

STRONGYLUS (DELAFONDIA) VULGARIS (Looss, 1900).

This Strongyle was still more numerous than the two preceding species ; it was restricted to the cæca of the three cases examined.

These three species were so common that it was not possible to count them all. Six hundred specimens were counted from one horse only ; and still larger numbers were collected but not counted.

TRIODONTOPHORUS SERRATUS (Looss, 1900).

Four specimens of this parasite were found in the large colon of one of the horses. None were obtained from the other two.

TRIODONTOPHORUS BREVICAUDA Boulenger, 1916.

This species was exceedingly rare. Only two specimens were found in the cæcum of one horse. None were found in the other horses.

TRIODONTOPHORUS TENUICOLLIS Boulenger, 1916.

This species was collected from the three horses. It was found in the right dorsal colon from which 36 specimens were collected.

Genus *TRICHONEMA* Cobbold, 1874.

Subgenus *TRICHONEMA* le Noux, 1924.

TRICHONEMA (TRICHONEMA) ÆGYPTIACUM Railliet, 1923.
Syn. *T. tetracanthum* Mehlis of Looss, 1900.

This parasite was not very numerous in any of the cases examined.

T. (TRICHONEMA) CORONATUM (Looss, 1900).

This species was found in the cæcum of the three horses, but it was not present in large numbers.

Subgenus *CYLICOSTEPHANUS* Ihle, 1922.

T. (CYLICOSTEPHANUS) CALICATUM (Looss, 1900).

A few specimens were obtained from the cæcum of two horses.

T. (CYLICOSTEPHANUS) LONGIBURSATUM (Yorke and Macfie, 1918).

This was the most common parasite found in the horses examined. It occurred in very large numbers in the cæcum and large colon.

T. (CYLICOSTEPHANUS) MINUTUM (Yorke and Macfie, 1918).

In one horse two specimens were found. None were found in the other two.

Subgenus *CYLICOCERCUS* Ihle, 1922.

T. (CYLICOCERCUS) GOLDI (Boulenger, 1917).

A common parasite of the cæcum and ventral colon of the three cases examined.

T. (CYLICOCERCUS) PATERATUM (Yorke and Macfie, 1919).

Three specimens were found in the cæcum of one of the horses.

T. (CYLICOCERCUS) ALVEATUM (Looss, 1900).

Three specimens of this parasite were obtained from the ventral colon.

Subgenus *CYLICOCYCLUS* Ihle, 1922.

T. (CYLICOCYCLUS) NASSATUM (Looss, 1900).

T. (CYLICOCYCLUS) NASSATUM (Looss) VAR. PARVUM (Yorke & Macfie, 1918).

This, along with the preceding species, was found in very large numbers in the ventral colon.

T. (CYLICOCYCLUS) INSIGNE (Boulenger, 1917).

A common parasite which occurred in very large numbers in the right dorsal colon.

T. (CYLICOCYCLUS) ELONGATUM (Looss, 1900).

Two specimens were found in the cæcum of one of the horses.

T. (CYLICOCYCLUS) RADIATUM (Looss, 1900).

One specimen was obtained from the cæcum of a horse.

Genus *CRATEROSTOMUM* Boulenger, 1920.

CRATEROSTOMUM MUCRONATUM (Ihle, 1920).

One specimen was obtained from the dorsal colon.

Genus *ŒSOPHAGODONTUS* Railliet and Henry, 1902.

ŒSOPHAGODONTUS ROBUSTUS (Giles, 1892).

Two specimens, from the right ventral colon.

REFERENCES.

BISSETT, N., 1925.—"Some Notes on Mountain Disease (Mountain Sickness—Clefyd-y-mynydd)." *The Welsh Journal of Agriculture*, vol. I., p. 48.

THEILER, G., 1923.—"The Strongylids and other Nematodes parasitic in the intestinal tract of South African Equines." pp. 5-81.

WALTON, C. L., 1924.—"Preliminary Report on the Agricultural Zoology of North Wales." p. 5.

Journal of Helminthology, Vol. IV., *Nos.* 4-5, *Nov.*, 1926, *pp.* 183-184.

A Further Note on *Hexatylus viviparus* Goodey, 1926.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Earlier in this volume (pp. 27–30), the writer has given an account of an eelworm obtained from a diseased potato tuber, possessing a mouth stylet with six basal swellings, an œsophagus without a muscular bulb such as occurs in *Tylenchus* and *Aphelenchus*, a vulva much more posterior in position than in the two genera just mentioned and a uterus without a post-vulvar diverticulum. On these characters the worms were placed in a new genus, *Hexatylus*.

The purpose of the present note is to put on record the occurrence of the worms in a diseased Gladiolus corm which was sent to the writer from the Ministry of Agriculture Pathological Laboratory, Harpenden. In the brown decomposing tissues of the corm the worms were present in large numbers and no other genus of nematode appeared to be present. Most of them were late larvæ and sexually immature adults ; comparatively few were fully grown mature adults. As in the case of the worms from the potato tuber, only females have been found ; males appear to be entirely absent. Careful teasing of material taken from the region where healthy and diseased tissues meet has failed to reveal the worms, which seem to be confined to the older decayed areas, and the conclusion has therefore been drawn that the worm is not the primary cause of the diseased condition.

Another point of interest is that the worms from the Gladiolus corm are longer than those originally described and are, on the whole, slenderer in appearance which may be due to the fact that very few have been found with the gonad fully mature.

Measurements.—Total length, 1–1·5 mm., greatest width, ·037–·057 mm., buccal stylet, ·01–·011 mm., anterior end to nerve ring, ·1–·12 mm., anterior end to excretory pore, ·155–·171 mm., vulva to tip of tail, ·16–·21 mm., anus to tip of tail, ·09–·1 mm.

The above measurements of adult worms are supplementary to those given in the original description which were made on specimens mounted in glycerine. Re-examination of the latter shows the measurements to be correct but that the worms, although adults, are quite small compared with those from the Gladiolus corm and small also in comparison with some of those from the potato tuber as revealed by the drawings in the original paper. In fact, in going over these again there is an apparent discrepancy between the measurements set down and those obtained by the use of the scales accompanying the drawings. The explanation of this is that the drawings were made from larger specimens freshly teased from the potato tuber and killed by gentle heat. These larger dimensions were unfortunately, by an oversight, not included in the principal measurements given and the opportunity afforded by the present note is therefore taken for the needed explanation.

Detailed examination of fresh and fixed specimens under high magnification shows that the worms from the Gladiolus corm have the same structure as those found in the potato tuber and there is practically nothing to add to the original description. One or two points may, however, be noted. At the base of the stylet there appears to be a small diverticulum, dorsal in position, closely approximated to the lumen of the œsophagus. It is probably the outlet of the dorsal œsophageal (salivary ?) gland. The writer has seen a similar structure in *Tylenchus dipsaci* and *T. hordei*. At a point about midway between the base of the stylet and the nerve ring, two very fine ducts have been observed opening into the lumen of the œsophagus, ventral or sub-ventral in position. These are taken to be the outlets of the sub-ventral œsophageal glands.

With regard to the gonad, the view was expressed in the original paper that the worms were viviparous. This was based on the observation that embryonated eggs were seen in the uterus of one or two worms together with the fact that no free eggs were found in the teasings of diseased potato tissues. This requires some qualification for in the present case the writer has found a few eggs lying free in the teasings of the Gladiolus corm. As no other nematode appeared to be present these eggs were taken to be those of Hexatylus. They were in an advanced state of segmentation and in view of this observation it is quite possible that the worm is not really viviparous in habit.

Journal of Helminthology, Vol. IV., *Nos.* 4-5, *Nov.*, 1926, *pp.* 185-190.

On the Morphology of the Free Living Larvæ of *Chabertia ovina.*

By Thomas W. M. Cameron, M.A., B.Sc., Ph.D., M.R.C.V.S.

(*Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

Material.

Adult gravid females were collected from the large intestine of sheep, carefully washed and teased up in water. The grosser material was strained off and the eggs, together with the smaller particles, were cultured in excavated glass cells at 27° C.

The eggs hatched out in one to three days and the larvæ which emerged (first stage larva) grew and moulted within two days, the resulting form (second stage larva) being morphologically almost identical with the first. After a further average period of about two days, the larva moulted again but remained within its first larval sheath (the infective larva).

The First Stage Larva.

The first stage larva (Fig. 1) is when newly hatched about ·45 mm. long and about ·02 mm. broad. The cuticle is transversely striated. The longitudinal lateral lines may be observed as triangular in cross section. They are best seen in the cast cuticle. The tail is elongated and tapers gently from the region of the anus to a fine point. The mouth opening is surrounded by six mammillated papillæ, shewn diagrammatically in Fig. 2. The œsophagus, which is about ·1 mm. long is of the typical " double-bulbed " or rhabditiform shape. In the newly hatched larva, the buccal tube is collapsed and only seen with

difficulty but in a few hours it becomes normal in shape (Fig. 3). In optical section it shews as two straight parallel lines terminating posteriorly in two "dots." These dots are continuous with the cuticular lining of the œsophagus, the anterior portion of this cuticular lining being dilated to the diameter of the buccal tube. The muscular part of the œsophagus surrounds the posterior sixth of the buccal tube, but fine strands are directed forwards to enclose the posterior half of the tube.

The posterior part of the œsophagus, or "sucking bulb" contains a valvular apparatus (Fig. 4) to assist in the ingestion of food material. This valve consists of two sets of plates: an anterior set of hard cuticular curved plates (with the concave side anterior) and a posterior set of more elastic cuticular plates curved in the same direction. At the rest position, these plates are in apposition (Fig. 4A). When the larva wishes to swallow any material, the posterior set of plates are pulled backwards, by means of the muscles of the œsophagus, from the centre —the œsophageal-intestinal valves being closed. These plates are attached to the outer edge of the anterior plates, and their backward motion has the effect of moving these plates backwards and inwards, thus opening the lumen of the œsophagus (Fig. 4C). The posterior part of the valve is then slightly released, thus allowing the anterior plates to close the lumen of the œsophagus (Fig. 4D) and the food passes into the intestine, opening the œsophageal-intestinal valves as it does so. With the further release of the posterior portion of the valve, the valve returns to the rest position and the food is completely passed into the intestine. The œsophageal-intestinal valves now close and the cycle is repeated. An actively feeding larva repeats this process several times a second, for a few seconds, rests for some seconds and then commences again. In cold weather, the action is slower and may easily be observed under a high power objective.

At the junction of the œsophagus and intestine are three valves referred to above. The intestine consists of eight cells on either side of the lumen and terminates in a short tubular rectum at the anterior end of which are three rectal mother cells. The anus is about ·1 mm. from the posterior end and divides the body in the ratio of 3·5 : 1.

The genital rudiment is situated on the ventral side of the body at about the level of the middle of the intestine with which it is in close apposition.

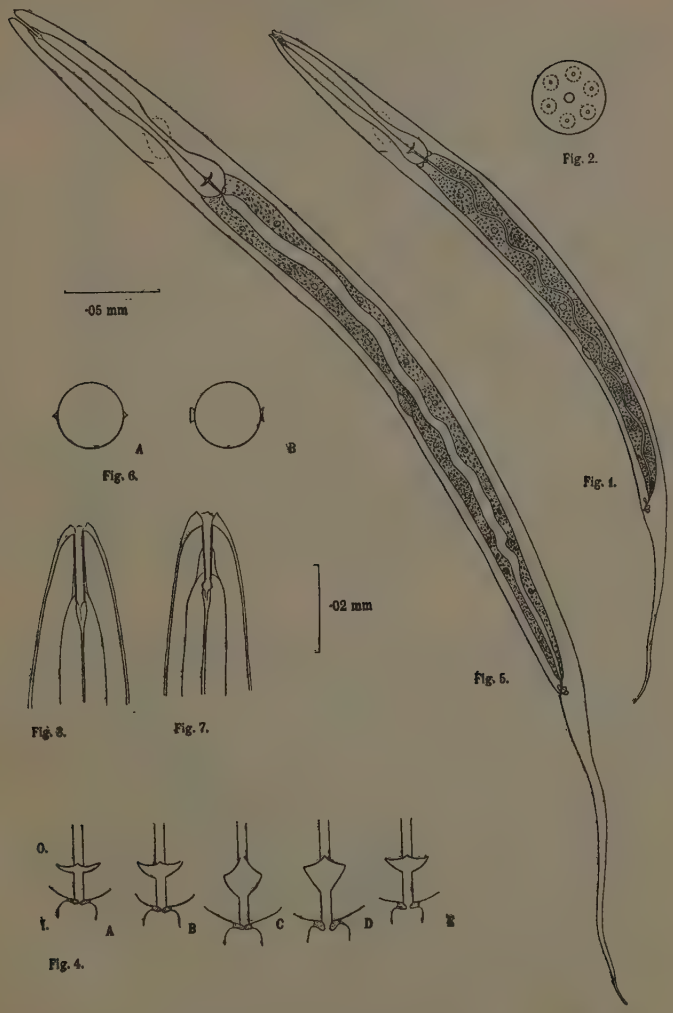


Fig. 1.—Newly hatched larva. Fig. 2.—Diagram of mouth papillæ. Fig. 3.—Head of larva, 15 hours old. Fig. 4.—Diagrams shewing action of œsophageal valves. Fig. 5.—Second stage larva. Fig.—Lateral lines of first and second stage larvæ. Fig. 7.—Head of second stage larva.

The nerve ring surrounds the constriction in the œsophagus in the normal position. The excretory opening is at the same level.

This stage rapidly grows and feeds but is not very resistant to external influences. It is easily killed by drying, by a temperature of about 50° C., and by being kept overnight in the ice chest.

The Second Stage Larva.

The first stage larva after feeding and growing, enters into a lethargus stage from which it emerges as the second stage larva (Fig. 5). This stage is about ·65 mm. long and ·03 mm. broad when it has newly emerged from its cast sheath. Apart from slight differences in size, and in other slight details, it very closely resembles the previous stage. It may be recognised by the fact that the lateral lines are double when viewed from the side, whereas in the first stage they are single. Their appearance is indicated in the diagram (Fig. 6). This is similar to the appearance observed by Looss in the larvæ of *Ancylostoma duodenale*. The buccal tube differs slightly from the first stage in that the posterior dots are replaced by distinct short curved rods (Fig. 7). The anus is ·17 mm. from the posterior end, thus dividing the body in the ratio of 4 : 1.

The Infective Larva.

The second stage grows, but not so quickly as does the first, and after a lethargus (Fig. 8) passes into the ensheathed infective larva (Fig. 9). The infective larva (including the sheath) is about ·65 mm. long and ·03 mm. broad; without its sheath the larva is about ·5 mm. long. The sheath preserves the outline of the previous stage, but the openings of the mouth, anus, and excretory pore are no longer patent. The cuticle of the larva has fine transverse striations on the cuticle and surrounding the mouth opening are six mammillated papillæ which are much less conspicuous than in the earlier stages. In the centre of these is a small mouth opening which leads by means of a fine, thinly cuticularised tubule to a complicated œsophageal armature (Fig. 10). This consists of a set of three cuticularised, triangular plates terminating the anterior end of the œsophagus. The apex of each triangle is directed forwards and is connected by means of a lightly cuticularised portion with the small buccal tube to form an inverted filter funnel. This is only produced after the lethargus during which the cuticle is cast off.

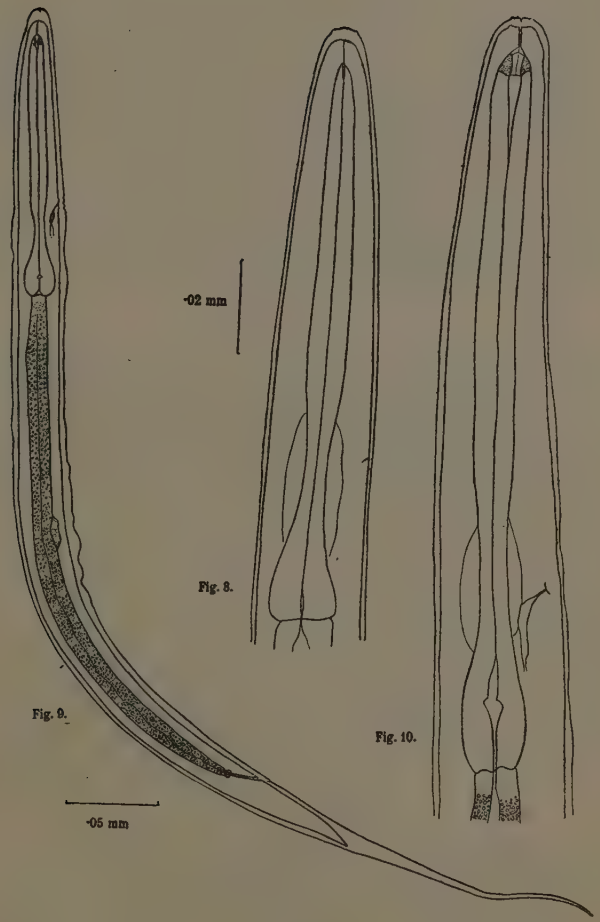


Fig. 8.—Head of larva in second lethargus. Fig. 9.—Infective larva (this is drawn to the same scale as Figs. 1 and 5). Fig. 10.—Head of infective larva.

The casting of the cuticle is not subsequent to the lethargus as in the previous stages but takes place during this phenomenon.

The œsophagus is less well divided into three portions although a distinct neck is still visible. The valvular apparatus has given place to an ill defined cavity in the posterior bulb. The intestine has now reached the 32-cell, stage but the division is not very regular and a few cells more or less than this number are occasionally seen. The rectum and the rectal cells are still obvious. The anus is about ·05 mm. from the posterior end of the body, thus dividing the body in the ratio of 9 : 1. The tail is short and conical, and forms a sharp point at the tip.

The genital rudiment is still seen at about the level of the middle of the intestine and although it has increased slightly in size it is still very minute.

The excretory pore is about the level of the nerve ring at the constriction of the œsophagus and is connected to a fairly large excretory vesicle which can be observed regularly filling and emptying, apparently into the space between the sheath and the larva. The vesicle has two ducts emptying into it, but the further course of these ducts could not be traced.

Journal of Helminthology, Vol. IV., *Nos.* 4-5, *Nov.*, 1926, *pp.* 191-198.

Some Stages in the Development of *Œsophagostomum dentatum* from the Pig.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Introduction.

In an earlier paper (Goodey, 1924), the writer has given an account of the morphology of the adults of *Œsophagostomum dentatum*, the development and structure of the free-living larvæ and the biology of the ensheathed infective stage. So far, however, the parasitic larval stages have not been described. In the present paper some of the gaps in our knowledge of the complete life-history of the worm represented by these stages are filled in, not as completely as could be desired owing to the absence from the material of specimens showing the 3rd and 4th ecdyses. The worms found, however, show that the 4th stage larva has a provisional buccal capsule essentially similar to that found in 4th stage larvæ of *O. radiatum* (Marotel, 1908) and *O. columbianum* (Veglia, 1924), and in this respect is in line with certain other 4th stage larvæ of the Strongyloidea such as *Ancylostoma duodenale* (Looss, 1897), and *Triodontophorus tenuicollis* (Ortlepp, 1925), in which provisional buccal capsules built on the same general plan have been observed.

The specimens were obtained on post-mortem examination of two pigs which had been fed from time to time with food containing ensheathed infective larvæ of *O. dentatum*. Six very small 3rd stage larvæ and a considerable number, about 50, 4th stage larvæ were picked out from the contents of the cæcum and large intestine after suitable sieving and sedimentation had been carried out. In no post-mortem

examination of a pig, even when showing a good infection with *O. dentatum*, has the writer seen any sign of intestinal nodules such as are produced by *O. columbianum* in sheep and goats and by *O. radiatum* in cattle. This being the case it would be of considerable interest to determine the characters of the worm said to produce nodules in the pig's intestine and described as *Strongylus follicularis* (Ostertag in Olt, 1898), assigned to *O. dentatum* in the Index Catalogue of Nematoda (Stiles and Hassall, 1920).

Third Stage Larvæ.

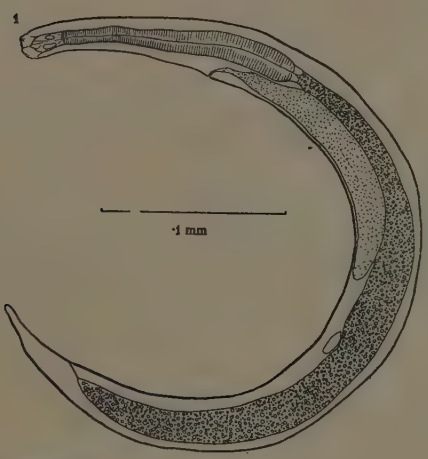
The few 3rd stage larvæ found are without sheaths and have undergone a certain amount of development. They have practically the same length as the larva lying within the sheath described in the earlier paper, *i.e.*, about ·55 mm., but are rather stouter in appearance and in most of them the body is strongly curved ventrally. The anterior end has become flattened as compared with the truncated conical head of the ensheathed larva. The excretory pore is rather prominent and the excretory duct leads inwards to a large granular mass reaching about half-way down the intestine, representing the developing cervical glands. The tail has not yet altered its shape but even among the few specimens available one or two seem to have rather longer tips than the others and this may perhaps be the first sign of sexual differentiation so well marked in the 4th stage larvæ.

In the previous paper particular attention was devoted to the anterior end of the œsophagus of the ensheathed larva where a complex network of fibres formed from branches of the cuticular lining of the œsophagus was found. In the 3rd stage larvæ from the pig the same structure is to be seen though the separate fibres composing it are not so distinctly discernible. In one or two cases there appear to be vacuoles in process of formation within it and these have an appearance which suggests that with their further growth and fusion an open cylindrical capsule will be formed. It is clear that the network of fibres is the groundwork from which the provisional buccal capsule of the 4th stage larva is built up though none of the worms shows the completely developed structure.

The œsophagus has assumed more the adult shape by thickening out in the region of the excretory pore where it was very narrow in the ensheathed larva. The intestine is very granular in appearance and calls

for no special description. The genital primordium occupies the same position as in the infective larva and has not yet increased in size.

An ecdysis separates this stage from the next but none of the examples collected show this interesting transitional condition.



Œsophagostomum dentatum.

Fig. 1.—Young 3rd stage larva showing formation of vacuoles in the structure at the anterior end of the œsophagus, the growing cervical glands and the unaltered genital primordium.

Fourth Stage Larvæ.

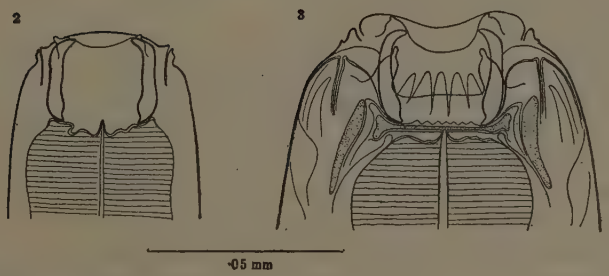
Of the numerous larvæ of this stage of development which were obtained some were obviously smaller and presumably younger than others. It is proposed to describe the most important characters of these first and then deal with the larger specimens which show features of the adult condition in process of development.

The smallest examples were two males measuring 2·32 mm. and 2·5 mm. and two females 2·97 mm. and 3·3 mm. in length. There is a marked difference in the tails of the two sexes ; the male tail tapers rapidly from the anus backwards and is short, whereas the female tail is long and tapers gradually. Many features in the worms of both sexes give them more the appearance of adults than of larvæ except that the males are without a bursa and both sexes are provided with a provisional buccal capsule. The cuticle is transversely striated, a cervical groove is present in the same position as in the adult and the duct from the cervical glands opens into it mid-ventrally. These glands are very large and extend backwards in the body to more than half its length. When viewed from the ventral or the dorsal aspect the very fine points of the cervical papillæ can be seen projecting through the cuticle in the same position as in the adults.

The anterior end is flattened and the central, terminal mouth is surrounded by a mouth collar separated from the body by a narrow cephalic groove. There are six circum-oral papillæ occupying the same position as in the adult, two laterals bearing the openings of the cephalic glands with slightly prominent lips, two sub-ventrals and two sub-dorsals which stand out a little from the surface of the mouth collar. Each of the latter is mammilliform in shape and is tipped with a nipple-like prominence.

The provisional buccal capsule is a comparatively large structure, about as broad as long, shaped rather like a short cylinder open at top and bottom. The walls, as seen in optical section, are thick and heavily cuticularised and have the shape shown in Fig. 2. Anteriorly each side narrows down to a rounded point projecting inwards whilst posteriorly after swelling out a little there is a narrowing on the inside to a conical point directed inwards and resting on the anterior end of the œsophagus. Projecting into the floor of the buccal cavity on the dorsal side there is a small pointed tooth which carries the opening of the duct from the dorsal œsophageal gland. The œsophagus is club-shaped as in the adult and is crossed by the nerve ring at about the level of the cervical groove. The intestine and rectum are also adult in appearance and the three rectal cells are prominent in both sexes in the vicinity of the junction of the intestine with the rectum.

Male Characters.—In the smallest males there appear to be two groups of cells somewhat pyriform in shape, lying dorsally to the rectum, which represent the beginnings of the spicules, accessory piece and telamon. In rather older and larger specimens these cell masses have grown forwards and become narrower whilst in one still older they can



Œsophagostomum dentatum.

Fig. 2.—Young 4th stage larva, anterior end in dorsal view showing provisional buccal capsule, lateral and sub-dorsal circum-oral papillæ and dorsal tooth.

Fig. 3.—Older 4th stage larva, anterior end in dorsal view showing provisional buccal capsule, lateral and sub-dorsal papillæ of larva with adult lateral and sub-dorsal papillæ just posterior to them. The developing adult buccal capsule with indications of the internal and external leaf crowns is also figured.

be seen as two long bands running dorsally to the intestine in the same position as the spicules in the adult worm. The various parts of the accessory piece and the telamon are not discernible at this stage of growth.

The genital primordium in the youngest male lies ventral to the intestine at a point about three-quarters of the length of the body from the anterior end and has grown to a strand of cells stretching posteriorly for a short distance. In the male, a little larger, this band of cells is longer and reaches further backwards whilst in the largest specimen it has reached the anus and has become tubular in its hindermost part to form the vas deferens and other regions of the genital duct. Anteriorly it remains solid and forms the testis.

In the largest male indications of the developing bursa can be seen under the cuticle dorso-laterally to the rectum, but the individual bursal rays cannot be distinguished.

Female Characters.—In the young female specimens the developing genitalia have already become double and the beginnings of the ovejector apparatus occupies its adult position and has the same general shape as that found in the adult worms. The pars ejectrix lies parallel to the longitudinal axis of the body and at each end is connected to a pars haustrix which is solid in appearance in the youngest forms found but in slightly older ones has become tubular. At the middle of the pars ejectrix the orifice leading to the growing vagina can be seen. The vagina appears to be formed from a mass of cells, probably ectodermal in origin, lying immediately ventral to the pars ejectrix. At first it is solid in appearance but soon becomes hollow and joins up with the centre of the ventral face of the pars ejectrix. Each pars haustrix is connected anteriorly with a narrow band of cells which gradually increase in length and breadth and are destined to become the ovaries and uteri.

Development of Adult Head Characters.

The researches of other workers have shown that in the case of those 4th stage Strongyle larvæ whose development has been studied there comes a time in the growth of the anterior end when vacuoles and spaces make their appearance in the vicinity of the provisional buccal capsule. In these spaces the definitive buccal capsule and the other structures characteristic of the adult head are to be seen so that when the final ecdysis comes the adult features are revealed. The same holds true of *O. dentatum.* Round the base of the provisional buccal capsule, in the angle formed by its outer border with the end of the œsophagus, a space

makes its appearance in which the material of the definitive buccal capsule is laid down. At the same time spaces are formed around the middle of the provisional buccal capsule within which the adult circum-oral papillæ develop. The appearance of the anterior end of a larva in which the various features of the adult head are in an advanced stage of development is shown in Fig. 3. This is a camera lucida drawing of a dorsal view as seen under the oil immersion and shows the lateral and sub-dorsal papillæ of the 4th stage larva with the corresponding lateral and sub-dorsal papillæ of the adult head immediately behind them.

At the base of the capsule there is a ring of cuticularised material which is the developing adult buccal capsule. On its anterior border one can discern, by carefully focusing, the rudiments of the internal leaf-crown in the form of a fairly well defined dentation of the edge. Very faint indications of the larger triangular elements of the external leaf crown have also been seen, but owing to the presence of debris within the larval buccal capsule the outlines of these structures are very indistinct.

The exact shape of the material at the outer angle between the provisional buccal capsule and the œsophagus is very difficult to determine in optical section owing to the fact that one is focusing through a considerable thickness of material. The ring of the final buccal capsule appears to be hollow and the anterior margin is raised up in such a way that when seen in optical section from any aspect it appears at the edge to be in advance of the rest of the ring. The developing capsule figured has not reached its final condition but it is not difficult to visualise from it the production of the adult buccal capsule having the shape figured in the writer's previous paper. This final condition would no doubt be revealed by a specimen showing the last ecdysis but unfortunately such a form has not been found. The chief features of the adult head structures have, however, been made out in the larva figured and it may be possible to fill in minor details from later findings of developmental stages of the parasite.

References.

Goodey, T., 1924.—" The Anatomy of *Œsophagostomum dentatum* (Rud.), a Nematode Parasite of the Pig, with Observations on the Structure and Biology of the free-living Larvæ." *J. Helm.*, 2, pp. 1-14.

Looss, A., 1897.—" Notizen zur Helminthologie Egyptens—II." *Cent. f. Bakt.*, Abt., Bd. xxi., pp. 913-926.

Marotel, G., 1908.—" L'Œsophagostomose nodulaire." *J. de Méd. Vet. et de Zootechnie*, 12, pp. 522-534, 591-604.

Olt, 1898.—" Die entozoischen Follikularerkrankungen im Darme des Schweines." *Zeitschr. f. Fleisch- u. Milchhyg.*, Berl., v. 8 (7), pp. 121-123.

Ortlepp, R. J., 1925.—" Observations on the Life History of *Triodontophorus tenuicollis*, a Nematode Parasite of the Horse." *J. Helm.*, 3, pp. 1-14.

Veglia, F., 1924.—" Preliminary Notes on the Life History of *Œsophagostomum columbianum*." 9th and 10th Reports of the Director of Vet. Ed. and Res., Union of S. Af. Dept. of Agric., pp. 811-823.

Journal of Helminthology, Vol. IV., *Nos.* 4-5, *Nov.*, 1926, *pp.* 199-202.

On the Identity of *Physaloptera caucasica* v. Linstow, 1902, and *Physaloptera mordens* Leiper, 1908.

By R. J. Ortlepp, M.A., Ph.D., F.Z.S.

(*Senior Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Two species belonging to the Nematode genus Physaloptera have up to the present been reported from Man; *P. caucasica* v. Linstow, 1902, which has been obtained on one occasion only from the Caucasus, and *P. mordens* Leiper, 1908, which has been reported on several occasions from Man and Monkeys from Central Africa. Until quite recently the only account of the former species was that of v. Linstow, and from this it was quite plain that the two species were distinct. Schulz (1926), however, has had the opportunity of re-examining the types of *P. caucasica* and on the basis of his excellent descriptions and figures it is possible to make a closer comparison between it and *P. mordens*. Schulz was able to rectify several inaccuracies in v. Linstow's description, and he came to the conclusion that the two species were very closely related, the only difference between the two being that in *P. caucasica* there is in addition to the three large teeth on each lip also a series of small teeth between and exterior to them on the inner face of the lip, which teeth are absent in *P. mordens*; also that the former species was smaller than the latter, 14-24 mm. to 29-34 mm. for the male and 24 mm. to 41-100 mm. for the female respectively, and that the eggs of the former were larger.

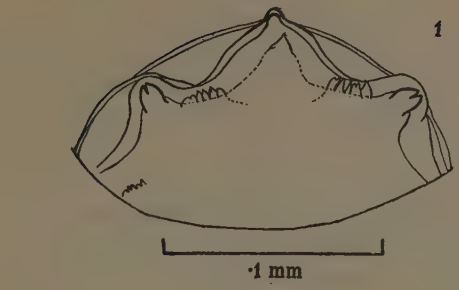
In consequence of this description Prof. R. T. Leiper requested the writer to re-examine the types of his species as well as the other specimens of *P. mordens* collected from Man and Monkeys contained in the

Helminthological Collection of the London School of Hygiene and Tropical Medicine, and see whether these small teeth are really absent, and, if not, whether the species are co-specific or not.

In 1922 the writer gave a re-description of *P. mordens* based on part of the above mentioned material, and a drawing of the inner view of the lip was included which showed that the small teeth were absent. This lip was obtained by cutting off the head and separating the lips from one of the worms collected from man. Unfortunately no permanent preparation was made of these lips. The writer has in consequence repeated this procedure, sacrificing one of the type males and two other specimens collected from man ; in addition specimens obtained from monkeys were similarily examined. The result of this examination showed that the small teeth were present on all the lips so examined (Figs. 1, 2 and 3), although they were not seen when the lips were examined prior to their being removed from the worms. The fact that in his first examination the lips were cleared and examined in creosote, whereas in the latter they were cleared in glycerine, probably explains why the writer missed them. The writer has now compared the effects of creosote and glycerine and finds that with creosote, whose refractive index is much higher than that of glycerine (1·538 and 1·473 respectively), the lips become so transparent that the small teeth can be noted only with difficulty and can thus be very easily missed ; this, however, is not the case with glycerine, especially if the glycerine has previously been diluted with an equal volume of 70 per cent. alcohol.

From Schulz's description and figures it appears that in the specimens examined by him the small teeth are regularly arranged, of more or less the same size, and he states that there are about 54 to each lip. The writer, however, finds considerable variation in all these characters ; *e.g.*, the teeth may be absent on some part of the lip where on the corresponding part of the other lip they are present ; the number on one half of the lip may not be the same as that on the other half ; the teeth may vary in size from mere serrations to well defined structures, these extremes being sometimes found on the same lip ; also the number for each lip was found to vary from 12 to 43.

The next point for consideration is the difference in size. Among the above mentioned material, part of which has been collected since the



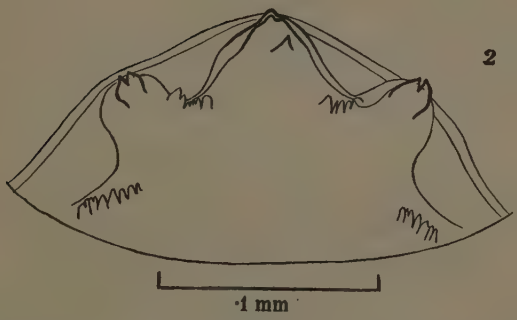


Fig. 1.—Inner view of lip of *Physaloptera caucasica*, one of the type males of *P. mordens*.
Fig. 2.—Inner view of lip of female obtained from man.

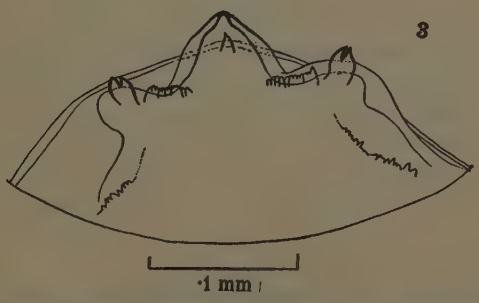


Fig. 3.—Inner view of lip of female obtained from *Papio homadryas*.
All figures to shew presence of small accessory teeth, and the variations in their size and number.

writer's account of *P. mordens* appeared, mature specimens have been obtained which for the female were only 22 mm. in length; much smaller specimens of apparently mature males were also present. There is thus for this species a range of variation for mature forms collected from Africa ranging for the female from 22 mm. to 100 mm. The sizes given for *P. caucasica* easily fall into the range of sizes of the above species.

With regard to the size of the eggs, Schulz found them to be from 0·057-0·065 mm. in length by 0·042-0·045 mm. in breadth for v. Linstow's species. In the writer's first description the sizes for those of *P. mordens* was given as being 0·045-0·049 mm. in length by 0·032-0·034 mm. in breadth; the writer has now examined a larger series and finds that they vary from 0·044 mm. to 0·051 mm. in length by 0·032 mm. by 0·039 mm. in breadth. This, however, is still considerably below the sizes given for the former species.

The question now arises whether the differences between the small teeth taken in conjunction with the difference in size of the eggs is sufficient to warrant keeping these two species separate. When one takes into consideration the fact that the reproductive systems in these two forms is now known to be the same; that the arrangement of the caudal papillæ on the tail of the male is on exactly the same plan; that the relative lengths of the œsophagus and the relative positions of the vulva is practically the same for both; then the writer considers that the difference in size of the eggs, and in the size and arrangement of the small labial teeth are to be considered as of only minor importance and must be regarded as variations which may possibly arise in any member of a given species due, perhaps, to different host reactions or other causes. In consequence, therefore, the name *P. mordens* Leiper, 1908, becomes a synonym of *P. caucasica* v. Linstow, 1902.

References.

Ortlepp, R. J., 1922.—"The Nematode Genus Physaloptera Rudolphi." *Proc. Zool. Soc.*, Dec., pp. 1052-1055 and 1089-1090. London.

Schulz, R. Ed., 1926.—"Sur la Morphologie du *Physaloptera caucasica* von Linstow, 1902, de l'Homme." *Ann. de Parasit.*, vol. IV., pp. 74-84. Paris.

Journal of Helminthology, Vol. IV., *Nos.* 4-5, *Nov.*, 1926, *pp.* 203-7

On the Round Worm Genera *Protostrongylus* and *Angiostrongylus* of Kamensky, 1905.

By R. T. Leiper, M.D., D.Sc., F.R.S.

(*Professor of Helminthology in the University of London, and Director of the Department of Helminthology in the London School of Hygiene and Tropical Medicine.*)

In "An account of some helminthes contained in Dr. C. M. Wenyon's collection from the Sudan," published in the Third Report of the Wellcome Research Laboratories at Khartoum, I drew attention in 1908 to the importance of the mouth armature of the Bursate Nematodes and suggested the regrouping of the various genera into three separate families : *Strongylidæ*, *Metastrongylidæ* and *Eustrongylidæ*. The *Strongylidæ* were divided into three and the *Metastrongylidæ* into two sub-families. Under the sub-families were listed the names of those genera which I then thought fell into each group, but to none did I append the name of the author or the date of the genus. The genus *Protostrongylus* occurred in the list of genera of *Metastrongylinæ*. Where, as in *Trichostrongylinæ* and *Metastrongylinæ* new names were suggested, these were indicated by the insertion of N.N. after each. Yet in two important compilations dealing with the classification of the Nematodes published during the present year the authors place on me the responsibility for proposing the generic name "*Protostrongylus*." Thus in Baylis and Daubney, page 253, the name is listed *Protostrongylus* Leiper, 1908, and it is stated that "this name is mentioned by Leiper among genera attributed to his sub-family *Metastrongylinæ*. No species is mentioned and the name appears to be a nomen nudum." Yorke and Maplestone similarly listed the genus *Protostrongylus* Leiper, 1908, among those genera insufficiently known and of uncertain systematic position, stating that it is one "belonging to the sub-family *Metastrongy-*

linæ. No species is mentioned and no description given." I have recently written to the senior authors of both of these volumes pointing out that the generic name *Protostrongylus* has been erroneously attributed to me and was published by Kamensky in 1905 in a paper entitled "The Systematic Position of the Genera *Metastrongylus*, Wost. and *Protostrongylus* g.n. among the other Strongylidæ" and that the name was listed and the title quoted in the Zoological Section of the International Catalogue of Scientific Literature for 1907.

The possibility however, that the name *Protostrongylus* might have priority over other names now coming into general use in the important groups of lung-worm parasites, led me to take steps to secure a translation of Kamensky's paper which appeared in *Sborn. Trood. Charkov. Veterinar. Institut.*, 1905, and which is, apparently, unobtainable in the original in this country.

Recently Dr. A. Schaurenkoff of the Russian Institute of Tropical Diseases, Moscow, kindly prepared for us a verbatim translation of Kamensky's paper, together with tracings of the figures which accompany it. It is evident from this translation that Kamensky's contribution is an extensive and important one, and that previous workers have overlooked two important and valid genera : *Protostrongylus*, which is mentioned in the title of the paper, and *Angiostrongylus*, which is described in detail in the text. The latter generic name has been omitted not only from the International Catalogue and the Zoological Record, but also from the volume dealing with Round Worms in the Index Catalogue of Medical and Veterinary Literature published by Stiles and Hassall.

A considerable part of Kamensky's paper is devoted to the discussion of the synonymy of the lung worms of sheep and hares. He points out that if the lung parasites of the hare are identical with those of the sheep the question is one of great practical importance. For *Strongylus rufescens* Leuckart, 1865, *S. commutatus* Diesing, 1851, and *S. pusillus* Müller, 1890, he forms a separate genus *Protostrongylus* for which he gives the following diagnosis :—

Protostrongylus g.n.

Corpus filiforme vel capillare, utrinque attenuatum aut æquale. Os terminalis margine papillis tribus bifidis minus vel vix conspicuis

instructo. Extremitas caudalis maris inflexa, aut convoluta, feminæ arcuata aut inflexa, utrique dorsaliter subcarinata, feminæ ventraliter toro præanale plusminusve instructa, apice mucronata. Spicula modo eorum g. Metastrongyli instructa, sed breviora, retorta, partibus accessoriis tribus (vel 2 ?). Vulva ani propinqua. Ovipari. Embryonis cauda undulata. Parasiti pulmonum, tracheæ et bronchium mammalium (herbivorum carnivorumque).

The genus is clearly different from *Metastrongylus* and the differences are set out in a table as follows :—

	Metastrongylus.	Protostrongylus.
Oral papillæ	6 single well seen	3 double, scarcely seen
Spicules	Very long, hair-like Spic. capillaria	Comparatively short and broad, rolled up near the middle—Spic. retorta or arch-like—Spic. arcuata
Ratio of length of the spicules to length of vagina	Greater than 1	Less than 1
Accessory pieces	1 small	3 (2 ?) different
Method of propagation	Ovoviviparous	Oviparous
Posterior end of the embryo	Sharp or mace-shaped	S-shaped or undulating
Hosts	Omnivora, rarely herbivora	Herbivora, rarely carnivora

Kamensky deals with the question of the identity of *S. rufescens* Leuckart, and *S. commutatus* Diesing, by means of a table of synonyms. It is evident that the lung worm of sheep is regarded by him as distinct from the species in the hare, and it is to be noted that he identifies *Filaria terminalis* Passerini, 1884, as a species of *Protostrongylus*. This synonymic table is as follows :—

Sheep. Protostrongylus rufescens (Leuckart, 1865).	Hares. Protostrongylus terminalis (Passerini, 1884).
S. retortæformis Rud. sp. maj. ex parte, 1819.	*S. retortæformis* Rud. sp. maj. ex parte, 1819.
S. commutatus Dies. ex parte, 1851.	*S. commutatus* Dies. ex parte, 1851.
S. rufescens Leuck., 1851.	*S. commutatus* Schn. ex parte, 1866.
S. commutatus Schn. ex parte, 1866.	*Pseud. ovis pulmonalis* A. Koch ex parte, 1883.
Pseud. ovis pulmonalis A. Koch ex parte, 1883.	*Filaria terminalis* Passerini, 1884.
S. rufescens Koch ex parte, 1883.	*S. commutatus* A. Müller, 1889.
S. sagittatus A. Muller, 1891.	*S. capillaris* M. Schleg., 1899.
S. capillaris M. Schl., 1899.	*S. rufescens* Raill. ex parte, 1895.
S. rufescens A. Raill. ex parte, 1895.	*S. commutatus* Raill. ex parte, 1895.

The selection however of the specific name *terminalis* for the lung worm of hares does not conform with the law of priority. *S. commutatus* Diesing, 1851, appears to be a specific name including two distinct forms, one in the hare and the other in sheep. For the lung worm in sheep the name *S. rufescens* of 1865 was made by Leuckart. It follows therefore that the name *S. commutatus* remained as the valid name for the lung worm of hares. In this restricted sense *S. commutatus* Diesing, 1851, is still available and cannot be displaced by the later *Filaria terminalis* Passerini, 1884.

Kamensky then proceeds to discuss the systematic position of two species of round worm recorded from the heart and pulmonary artery of the dog, viz., *S. vasorum*, Baillet, 1866, and *Ematozoa filaria cardiaca* Bozzi, 1870. These differ from the species of *Metastrongylus* in the absence of oral papillæ and of posterior rays in the male bursa, in reddish colour, and particularly in the twisted character of genital tubules around the intestine. On these grounds he believes that these forms must be isolated as a distinct genus *Angiostrongylus*. The genus thus includes *Angiostrongylus vasorum* (Baillet, 1866), and *Angiostrongylus cardiacus* (Bozzi, 1870).

After a discussion of the practicability of dividing the bursate nematodes into groups on the presence or absence of a chitinous mouth capsule, Kamensky proposed to unite the genera *Protostrongylus* and *Metastrongylus* into one sub-family *Protostrongylinæ*. For *S. filaria*, *S. viviparus*, *S. arnfeldi*, which at the present time are generally incorporated in the genus *Dictyocaulus* Railliet and Henry, 1907, he proposed the sub-family name *Blastostrongylinæ*, without however specifically creating the generic name which would properly have formed the type root for this new sub-family. Had a generic name been made by him for *S. filaria* and allied species it would have had priority over *Dictyocaulus*.

As *S. commutatus* is the type of *Synthetocaulus* Railliet and Henry, 1907, *Synthetocaulus* must now be suppressed in favour of *Protostrongylus*. The sub-family *Metastrongylinæ* Leiper, 1908, must give place to *Protostrongylinæ* Kamensky, 1905. The name *Metastrongylidæ* must therefore disappear as a family name and be replaced by *Protostrongylidæ* n.n. Moreover as *S. vasorum* is type of *Hæmostrongylus* Railliet and Henry, 1907, this latter generic name must give way to *Angiostrongylus*.

In the concluding paragraphs the author, in the course of a discussion on the evolution of round worms, refers incidentally to a sub-family *Angiostominæ*. This name, however, may be regarded as still-born, as *Angiostoma* falls into the synonymy of *Rhabdias ;* according to Stiles and Hassall.

Finally the genera *Eustrongylus*, *Hystrichis*, and *Gnathostoma* are placed by Kamensky in a separate family "*Eucosomidæ*." Like his sub-family "*Blastostrongylinæ*" this family is not based upon one of the genera which he includes in this grouping and is therefore invalid under our present procedure in nomenclature.

Index to Vol. IV.

Index of Authors.

PORTRAIT.

PROFESSOR FUHRMANN: an intimate study in his laboratory, Neuchatel.

Professor Fuhrmann recently spent some time working in the Institute under the auspices of the League of Nations.

CORRIGENDA FOR VOL. IV.

Page 8, line 4, for *Echinostomum* read *Echinostoma*.
Page 9, line 2, for *ancanthus* read *acanthus*.
Page 12, line 8, for *oligartha* read *oligarthra*.
Page 19, Fig. 2, line 4 of legend, for *c*, *d*, and *g*, read *c* and *d*.
Page 75, line 9 from bottom, for *parasite* read *parasitic*.
Page 86, line 1, for wel read well.

NEW NAMES IN VOLUME IV.